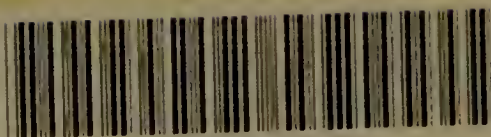




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# Laboratory Text-Book for Brewers.

BY

LAWRENCE BRIANT, F.C.S., F.R.M.S.

THIRD EDITION,

EDITED BY

HAROLD HARMAN.

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NEWCOMEN STREET, BOROUGH, S.E.

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## PREFACE TO THIRD EDITION.

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The second edition of the Laboratory Text-Book having become exhausted, and a new issue overdue, the author is glad to have secured the services of Mr. Harold Harman to revise and bring up to date the present edition.

The accusation has often been made against British manufacturers that they are slow to take advantage of the assistance which science can offer. The sister industries of brewing and malting are not, however, open to that charge, for the progress in the application of science to their technical operations has been remarkable, and may well be a just cause for congratulation, not the less because such has been rendered possible largely as the result of work by the chemists of this country.

The author is grateful if, since its first publication about 24 years ago, this little book has contributed to some small extent to the attainment of that result.

LAWRENCE BRIANT.

*March, 1911.*



## INTRODUCTORY NOTE.

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The book has necessarily been largely re-written and much new matter added.

As far as possible, however, the original character and aim of the book has been preserved, this being, as stated in the preface of the first edition, "to describe in as clear and concise a manner as possible, first, the composition and properties of those bodies entering into the constitution of materials used in brewing, and secondly, their determination by analytical means."

It will be recognised that in a book dealing almost entirely with analytical methods, such matter as is dealt with in Chapters II and XIII must be to some extent inadequate and incomplete. There is obvious need for expanding the subjects therein dealt with, so far as they are directly concerned with brewing, and the editor is hopeful of being able to publish at an early date a small companion book to the present work dealing more fully with this subject.

HAROLD HARMAN.

THE LABORATORY,  
24, HOLBORN VIADUCT, E.C.



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## CHAPTER I.

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### APPARATUS AND MANIPULATION.

IN order that the analytical experiments hereafter detailed may be successfully conducted, it is necessary that some points should first be dealt with, the consideration of which will materially assist us in subsequent work. A knowledge of the principles underlying the operations is requisite, together with an acquaintance with the construction and manipulation of the various instruments and apparatus which we shall require to employ.

It is not here proposed to explain general chemical principles—these may be studied in one of the numerous excellent books on the subject—but some little space may be devoted to the consideration of indispensable preliminaries, with brief explanations as to their bearing upon actual experimental work.

**Qualitative and Quantitative Analysis.**—Analysis is broadly divided into two classes, qualitative and quantitative, the former being that branch of practical chemistry which treats of the processes for detecting the constituents of a substance, whilst the latter deals with the determination of the exact weight of such constituents. The first-mentioned form of analysis necessarily precedes the latter, for obviously we must first know what are the bodies

present in a substance before we can estimate their proportions.

In many cases, however, the student of brewing chemistry will find a preliminary qualitative examination unnecessary, since it frequently occurs that he already knows what particular bodies are present, and merely wishes to ascertain their relative proportions.

The methods of quantitative analysis are conveniently divided into two classes—gravimetric and volumetric. By means of the former, we endeavour to weigh the known constituents of any substance, either in an elementary condition, or in some form of combination, the composition of which is definitely known. On the other hand, volumetric analysis seeks to estimate the amount of a substance from the determined action of reagents in solution of a known strength; the amount of the reacting substance being calculated from the volume of liquid used. To give simple illustrations of these two methods:—The chlorine in salt may be determined gravimetrically by addition to an acidified solution of an excess of silver nitrate, filtering, washing, and weighing the precipitate as silver chloride, and from the weight obtained calculating that of the chlorine; or it may be estimated volumetrically, when a standard solution of silver nitrate is added to the liquid, until, by means of a suitable indicator, we ascertain that all chlorine has been precipitated. Now, as we know the strength of our solution, and the amount of chlorine that each cubic centimetre (or a known volume) is capable of precipitating, it is easy to see that if we ascertain the volume of the silver solution required to precipitate an unknown quantity of chlorine in the liquid

under examination, the amount present may be arrived at by a simple calculation.

Volumetric processes generally possess the advantage of rapidity, combined with a fair, and sometimes great, degree of exactness. Each of the two branches referred to possesses its own particular merits and its own sources of inaccuracy, and these will be referred to as they occur.

### The Decimal System of Weights and Measures.

—In carrying out the operations of weighing and measuring in the laboratory, we must select a standard of comparison or unit of weight by which to work. The selection of this is merely arbitrary, and there are several different systems in use; there exist broad principles, however, which should guide us in the choice of a standard for scientific work.

1.—The unit should be moderately small.

2.—It should bear a simple relation to the other measures such as capacity and length.

3.—The larger and smaller weights should be derived from the unit by the most simple system of multiplication and division.

The above conditions are altogether insufficiently fulfilled by our own English standard, but are well adhered to in the French or metric system, and for this reason this latter is rapidly superseding all others in scientific work, and is now almost universally adopted in chemistry.

The foundation of the system is its unit of length, a *metre*, and this was arranged to be exactly one-forty-millionth of the earth's circumference.

Unfortunately, however, the measure when prepared was

found to be slightly incorrect, in fact, 0·0000086 metre too short. This, however, does not in any way impair its use as a scientific standard.

From this definite standard others are derived by merely multiplying and dividing it by 10, 100, 1,000, the names of the larger measures or multiples being obtained by prefixing to the word "metre" a numeral derived from the Greek; the names of the smaller measures by prefixing in a similar way numerals derived from the Latin.

In order that there may be no uncertainty as to the system, a table containing the standards of length, volume, and weight is subjoined.

#### LENGTH.

|                  |     |     |     |     |                 |
|------------------|-----|-----|-----|-----|-----------------|
| Kilometre        | ... | ... | ... | ... | = 1,000 metres. |
| Hectometre       | ... | ... | ... | ... | = 100 „         |
| Decametre        | ... | ... | ... | .   | = 10 „          |
| Metre (m.)       | ... | ... | ... | ... | = 1 metre.      |
| Decimetre        | ... | ... | ... | ... | = 0·1 „         |
| Centimetre (cm.) | ... | ... | ... | ... | = 0·01 „        |
| Millimetre (mm.) | ... | ... | ... | ... | = 0·001 „       |

#### VOLUME.

|   |     |     |     |     |                 |
|---|-----|-----|-----|-----|-----------------|
| Kilolitre                               | ... | ... | ... | ... | = 1,000 litres. |
| Hectolitre                              | ... | ... | ... | ... | = 100 „         |
| Decalitre                               | ... | ... | ... | ... | = 10 „          |
| Litre                                   | ... | ... | ... | ... | = 1 litre.      |
| Decilitre                               | ... | ... | ... | ... | = 0·1 „         |
| Centilitre                              | ... | ... | ... | ... | = 0·01 „        |
| Millilitre (or cubic centimetre) (c.c.) |     |     |     |     | = 0·001 „       |

## WEIGHT.

|                 |     |     |     |                  |
|-----------------|-----|-----|-----|------------------|
| Kilogramme ...  | ... | ... | ... | = 1,000 grammes. |
| Hectogramme     | ... | ... | ... | = 100 „          |
| Decagramme ...  | ... | ... | ... | = 10 „           |
| Gramme (grm.)   | ... | ... | ... | = 1 gramme.      |
| Decigramme ...  | ... | ... | ... | = 0.1 „          |
| Centigramme     | ... | ... | ... | = 0.01 „         |
| Milligramme ... | ... | ... | ... | = 0.001 „        |

From the metre—the unit of length—the unit of volume is simply derived. The latter is merely a cube, each side of which measures 1 decimetre, and the capacity of such a cube we term a *litre*.

The other measures of capacity are obtained by multiplication and division by 10, 100, or 1,000, but it should be noted that in ordinary chemical operations quantities smaller than a litre are usually expressed in cubic centimetres, or, for the sake of brevity, c.c., so that a tenth of a litre, instead of being termed a decilitre, is generally spoken of as 100 c.c.

Lastly, we come to the unit of weight, termed 1 *gramme*. This, in its turn, is derived from the unit of capacity by being the weight of 1 c.c. of distilled water at a temperature of 4° C., but for all practical purposes the c.c. is taken as the bulk occupied by 1 gramme of water at 60° F. The great advantage of such a simple system of weights and measures over our own unsymmetrical and incongruous English standards will be obvious, but though analyses are now almost always conducted on the metric

system, it is still usual to express some results, such as those of water analyses, in terms of grains per gallon.

Equivalents of Liquid Measures in Common and Metric Measures and Cubic Feet or Inches.—From Wahl and Henius.

| Measures.         | Cu. In. or Ft.  | British Measure. | Litres.  |
|-------------------|-----------------|------------------|----------|
| Puncheon ...      | 17·3294 cu. ft. | 108 gal.         | 490·7    |
| Hogshead ...      | 8·6647 „        | 54 „             | 245·343  |
| Barrel, beer* ... | 5·776 „         | 36 „             | 163·5684 |
| Kilderkin ...     | 2·8882 „        | 18 „             | 81·78    |
| Firkin ...        | 1·444 „         | 9 „              | 40·89    |
| Gallon, Imperial  | 277·274 cu. in. | 1 „              | 4·5435   |
|                   | 277·463 „       |                  | 4·5465   |
|                   | (new)           |                  |          |
| Quart ...         | 69·3185 „       | 1 qt.            | 1·1358   |
| Pint ...          | 34·6592 „       | 0·5 „            | 0·5679   |
| Litre ...         | 61·0254 „       | 0·8803 qt.       | 1        |
| Gill ...          | 8·6648 „        | 0·125 „          | 0·142    |

**Specific Weight or Gravity.**—Specific gravity may be defined as the relative weight of equal volumes of different substances. Water is universally accepted as the standard for liquids or solids, but air for gases or vapours. With these two latter we need not concern ourselves, but the methods for the determination of the specific gravity of the former will be described in some detail.

In taking the specific gravity of a liquid, we have only to ascertain the weight of any known bulk, and compare

\* The Irish barrel is equivalent to 32 gallons.

it with that of an equal bulk of water ; to do this we merely require to take any vessel of convenient size—the weight of which is known—and determine its increased weight, first, when filled with water, and secondly, when filled with the given liquid under examination. But as, by the well-known law of expansion and contraction, a definite bulk of liquid will occupy a space varying with its temperature, it is therefore necessary that this should be the same in both cases. Of course, any might be chosen, but in practical working it is found generally convenient to adopt one definite temperature for the determination of the specific gravity of all substances, liquid or solid, and a sufficient reason for this we shall immediately see, as we come to consider the best method for making such observations.

**Specific Gravity Bottle.**—Specific gravity is determined in the case of liquids in what is known as a specific gravity bottle, which may be described as consisting of a small flask, of a capacity of about 50 c.c., provided with a closely-fitting glass stopper, pierced through by a fine hole. By means of this stopper, the bottle may be exactly filled with any liquid, and stoppered without the enclosure of any air bubbles. For by filling it absolutely full, and gently pressing the stopper home, the excess of liquid escapes through its capillary hole, and the bottle is completely and accurately filled.

The specific gravity bottle is usually made to contain some precise quantity (very generally 50 c.c.), but as ordinarily purchased it is rarely correct. It may, however, be adjusted by filling with distilled water at 60° F. and



weighing ; then if holding, as is generally the case, less than the reputed amount, the bottom of the stopper is ground down by means of emery cloth, until enough has been taken away to correct the contents deficit. Should, however, the capacity of the bottle prove to be more than the reputed quantity, the stopper may be cautiously ground down round its sides, so that it fits lower into the neck of the bottle, thus reducing the contents capacity. As this, however, requires most careful management, for the grinding must be absolutely equal on all sides of the stopper if it is to fit perfectly, it is more advisable in such cases to employ the specific gravity bottle in its original uncorrected condition, since, if its precise capacity be known, a very simple calculation will give its comparison with water, the standard of which is usually taken as 1,000.

The bottle may be counterpoised by means of a hollow brass weight, with screw-on lid, which is adjusted by means of shot to the exact weight of the clean, dry bottle, or it may be counterbalanced by ordinary weights, a record of its tare being preserved and deducted from all subsequent calculations.

Great care must be taken that the bottle is not in any way chipped, or the results will become incorrect by reason of the alteration in its tare, and a blank experiment with distilled water should be frequently carried out to determine the correction for the bottle.

The determination of the specific gravity of a liquid is at the basis of many of the determinations afterwards to be made, and the student should have considerable practice in the manipulation.



To ensure accuracy, the following plan is recommended. Before filling the specific gravity bottle with the solution whose weight is to be determined, prepare a small bath of clean water (a 300-c.c. beaker will do quite well), and adjust the temperature of the water to  $60^{\circ}$ , very slightly above or below this reading according to whether the temperature of the air in the room is lower or higher than  $60^{\circ}$ .

Now rinse out the specific gravity bottle with a little of the solution at  $60^{\circ}$  whose specific gravity is to be determined. Suppose this to be a 10-per-cent. sugar solution. Afterwards fill the bottle completely and place it in the beaker-bath, the water level of which should be so adjusted as to be just below the opening of the bottle. Allow it to remain in the bath for a few moments, then rapidly push home the stopper, remove the bottle from the bath, and carefully wipe the capillary stopper *once*, after which the bottle itself is wiped and finally polished with a perfectly dry glass-cloth. In some cases it is very important to use a water-bath in this way, as the temperature of the outside air may be such as to alter the volume of the liquid in the specific gravity bottle with great rapidity, and so cause inaccuracy, owing to the bottle having been filled at a temperature either greater or less than  $60^{\circ}$ .

*Example.*—Suppose a perfectly clean and dry specific gravity bottle of 50 grammes capacity, accurately counterpoised or tared, and then filled with distilled water at  $60^{\circ}$ , as described above, and weighed.

This = 49.976 grammes, so that 0.024 gramme must be added as a correction to every subsequent weight obtained in gravity determinations.

Thus the 10-per-cent. sugar solution was found to weigh 51.620 grammes,  $\therefore 51.620 + 0.024 = 51.644$ , and this  $\times 20$ , to compare with  $1000 = 1032.88$ , the correct gravity of the 10-per-cent. solution.

**Hydrometer.** — The hydrometer furnishes another method for the determination of the specific gravity of liquids, and these instruments are almost exclusively used in actual brewing operations, and there are many varieties, but all are founded upon the same system, based upon the well-known principle of Archimedes, that when a solid is immersed in a liquid it loses a portion of its weight, the weight lost being equal to the weight of the liquid displaced by the solid, that is, to the weight of its own bulk of that liquid.

This statement affords the key to the general history of the equilibrium of floating bodies, of which an application is made in the common hydrometer. When a solid body is placed on the surface of a liquid specifically heavier than itself, it sinks down until it has displaced a body of liquid equal to its own weight, at which point it floats.

From this we see that if we place a hydrometer—the form of which is so well known that it needs no description—in a liquid whose specific weight is the same as its own, it will sink or float indifferently, remaining in the liquid at the level at which it is placed; but should the liquid be specifically heavier than itself, it will cease to sink when it has displaced a bulk of liquid equal to its own weight.

We employ, therefore, as a hydrometer a floating bulb of thin metal, or glass, having a weight beneath it to main-

tain it in an upright position, and a stem above bearing a scale suitably divided. The specific gravity of water is almost always taken as zero, the indication of the scale showing without any calculation the density of the liquid, as compared with that body.

These instruments may be used with a fair amount of accuracy, but it is most important that, in using any form of hydrometer, it should be tested against liquids of known gravity in order to ascertain whether the graduations are intended to be read off from the top, bottom, or centre of the meniscus, and, further, strict attention must be paid to the temperature at which such instrument has been graduated.

**Bates' Hydrometer** is such an instrument, its indication being directly in terms in excess of 1,000; but there are others quite as generally used in the manufactures, the scales of which, although based upon the specific gravity of water as a standard, yet vary in graduation, each instrument being suited more particularly to the liquid for which it is specially intended.

**Balling's Saccharometer** is graduated to indicate the percentage of dry solid matter in the liquid. It only, however, accurately shows the percentage when used for cane sugar solutions.

**Twaddell's Hydrometer** is very generally used in the arts and manufactures. This instrument is so graduated that real specific gravity may be deduced by a very simple method from the degrees indicated, viz., multiplying the latter by 5, and adding 1,000; the sum is the specific

gravity, water being 1,000. Thus 5° Twaddell indicates 1025 sp. gr.

**Baumé's Hydrometer** is in use on the Continent and also to a large extent in our own country. There are two different instruments of this name, one being for liquids heavier than water, and the other for those lighter.

To convert degrees Baumé into specific gravity, deduct the degree indicated from 144, and then divide 144 by the number so found and multiply by 1,000.

Thus, to convert 20° Baumé into specific gravity—

$$144 - 20 = 124$$

$$144 \div 124 = 1.16129$$

$$1.16129 \times 1000 = 1161.29 \text{ sp. gr.}$$

**Sykes' Hydrometer** is used by the Excise and Customs authorities, and the indications of this instrument are simply in degrees of specific gravity over the weight of water. Thus 25° Sykes corresponds to 1025 specific gravity.

**Brewer's or Long's Saccharometer** is, however, an instrument most commonly in use in the brewery, whose indications are based on a somewhat different—less commendable—system. Its indications are, as is well known, in “lbs. per brl.,” or in other words, in excess weight in lbs. over the normal weight of a barrel of water. Thus, one barrel containing 36 gallons weighs 360 lbs.—a gallon of water weighing 10 lbs.—and should a barrel of liquid weigh, say, 375 lbs., the indication by the saccharometer would be 15 lbs., that is the excess weight over the natural

barrel of water. Such, then, are the indications of "lbs. per brl.," somewhat clumsy indeed as compared with most other hydrometers, but almost universally used by English though but seldom by Scotch brewers.

The Excise authorities invariably deal with true specific gravity, so that the brewer must convert his own indications into this, which is easily accomplished by either multiplying by 2·77, or dividing by ·36. Reversely he may convert specific gravity degrees into his own saccharometer indications, by multiplying the excess weight over 1,000 by ·36, or dividing by 2·77.

Table for the Comparison of Different Saccharometers.

| Specific Gravity. | Brewers' Saccharometer, in Lbs. | Balling Degrees. | Specific Gravity. | Brewers' Saccharometer, in Lbs. | Balling Degrees. |
|-------------------|---------------------------------|------------------|-------------------|---------------------------------|------------------|
| 1000              | 0·00                            | 0·00             | 1019              | 6·84                            | 4·75             |
| 1001              | ·36                             | ·25              | 1020              | 7·20                            | 5·00             |
| 1002              | ·72                             | ·50              | 1021              | ·56                             | ·25              |
| 1003              | 1·08                            | ·75              | 1022              | ·92                             | ·50              |
| 1004              | ·44                             | 1·00             | 1023              | 8·28                            | ·75              |
| 1005              | ·80                             | ·25              | 1024              | ·64                             | 6·00             |
| 1006              | 2·16                            | ·50              | 1025              | 9·00                            | ·25              |
| 1007              | ·52                             | ·75              | 1026              | ·36                             | ·50              |
| 1008              | ·88                             | 2·00             | 1027              | ·72                             | ·75              |
| 1009              | 3·24                            | ·25              | 1028              | 10·08                           | 7·00             |
| 1010              | ·60                             | ·50              | 1029              | ·44                             | ·25              |
| 1011              | ·96                             | ·75              | 1030              | ·80                             | ·50              |
| 1012              | 4·32                            | 3·00             | 1031              | 11·16                           | ·75              |
| 1013              | ·68                             | ·25              | 1032              | ·52                             | 8·00             |
| 1014              | 5·04                            | ·50              | 1033·2            | ·96                             | ·25              |
| 1015              | ·40                             | ·75              | 1034·2            | 12·32                           | ·50              |
| 1016              | ·76                             | 4·00             | 1035·2            | ·68                             | ·75              |
| 1017              | 6·12                            | ·25              | 1036·3            | 13·04                           | 9·00             |
| 1018              | ·48                             | ·50              | 1037·4            | ·40                             | ·25              |

| Specific Gravity. | Brewers' Saccharometer, in Lbs. | Balling Degrees. | Specific Gravity. | Brewers' Saccharometer, in Lbs. | Balling Degrees. |
|-------------------|---------------------------------|------------------|-------------------|---------------------------------|------------------|
| 1038·4            | 13·76                           | 9·50             | 1070·0            | 25·21                           | 17·00            |
| 1039·4            | 14·12                           | ·75              | 1071·1            | ·61                             | ·25              |
| 1040·4            | ·48                             | 10·00            | 1072·2            | 26·00                           | ·50              |
| 1041·5            | ·84                             | ·25              | 1073·3            | ·39                             | ·75              |
| 1042·5            | 15·21                           | ·50              | 1074·4            | ·78                             | 18·00            |
| 1043·6            | ·58                             | ·75              | 1075·5            | 27·17                           | ·25              |
| 1044·6            | ·95                             | 11·00            | 1076·6            | ·56                             | ·50              |
| 1045·7            | 16·32                           | ·25              | 1077·7            | ·96                             | ·75              |
| 1046·7            | ·69                             | ·50              | 1078·8            | 28·36                           | 19·00            |
| 1047·8            | 17·07                           | ·75              | 1079·9            | ·76                             | ·25              |
| 1048·8            | ·45                             | 12·00            | 1081·0            | 29·16                           | ·50              |
| 1049·8            | ·83                             | ·25              | 1082·1            | ·56                             | ·75              |
| 1050·9            | 18·21                           | ·50              | 1083·2            | ·95                             | 20·00            |
| 1052·0            | ·60                             | ·75              | 1084·3            | 30·34                           | ·25              |
| 1053·0            | ·99                             | 13·00            | 1085·4            | ·73                             | ·50              |
| 1054·0            | 19·38                           | ·25              | 1086·5            | 31·12                           | ·75              |
| 1055·1            | ·77                             | ·50              | 1087·6            | ·50                             | 21·00            |
| 1056·2            | 20·16                           | ·75              | 1088·7            | ·87                             | ·25              |
| 1057·2            | ·55                             | 14·00            | 1089·8            | 32·25                           | ·50              |
| 1058·2            | ·94                             | ·25              | 1090·9            | ·64                             | ·75              |
| 1059·3            | 21·33                           | ·50              | 1092·0            | 33·04                           | 22·00            |
| 1060·4            | ·72                             | ·75              | 1093·1            | ·44                             | ·25              |
| 1061·4            | 22·11                           | 15·00            | 1094·2            | ·84                             | ·50              |
| 1062·5            | ·50                             | ·25              | 1095·3            | 34·23                           | ·75              |
| 1063·6            | ·89                             | ·50              | 1096·4            | ·63                             | 23·00            |
| 1064·6            | 23·27                           | ·75              | 1097·5            | 35·03                           | ·25              |
| 1065·7            | ·66                             | 16·00            | 1098·6            | ·43                             | ·50              |
| 1066·8            | 24·05                           | ·25              | 1099·7            | ·83                             | ·75              |
| 1067·9            | ·44                             | ·50              | 1100·8            | 36·23                           | 24·00            |
| 1069·0            | ·83                             | ·75              |                   |                                 |                  |

**Thermometers.**—Although the Fahrenheit thermometer scale is used almost exclusively in brewing, yet there is no doubt that the Centigrade, from its greater simplicity, is far preferable, and will gradually supersede the former; in fact, it is already very generally adopted in English



scientific work. In the Fahrenheit scale, the freezing point of water is placed at  $32^{\circ}$ , and the boiling point at  $212^{\circ}$ , the intermediate space being divided into  $180^{\circ}$ . On the Centigrade scale freezing point is placed at zero, boiling point at  $100^{\circ}$ , the interval being divided into  $100^{\circ}$ . The simplicity of this instrument must recommend itself to everyone, and it should undoubtedly, in point of merit, supersede our English scale. In some parts of Germany, and in Russia, there is another scale employed, namely, that of Réaumur, and in this the freezing point is fixed at  $0^{\circ}$ , and the boiling point at  $80^{\circ}$ .

### Conversion Tables of the Thermometer Scales.

| Fahren-<br>heit. | Centi-<br>grade. | Fahren-<br>heit. | Centi-<br>grade. | Fahren-<br>heit. | Centi-<br>grade. |
|------------------|------------------|------------------|------------------|------------------|------------------|
| °                | °                | °                | °                | °                | °                |
| 212              | 100              | 192              | 88·9             | 172              | 77·8             |
| 211              | 99·4             | 191              | 88·3             | 171              | 77·2             |
| 210              | 98·9             | 190              | 87·8             | 170              | 76·7             |
| 209              | 98·3             | 189              | 87·2             | 169              | 76·1             |
| 208              | 97·8             | 188              | 86·7             | 168              | 75·6             |
| 207              | 97·2             | 187              | 86·1             | 167              | 75·0             |
| 206              | 96·7             | 186              | 85·6             | 166              | 74·4             |
| 205              | 96·1             | 185              | 85·0             | 165              | 73·9             |
| 204              | 95·6             | 184              | 84·4             | 164              | 73·3             |
| 203              | 95·0             | 183              | 83·9             | 163              | 72·8             |
| 202              | 94·4             | 182              | 83·3             | 162              | 72·2             |
| 201              | 93·9             | 181              | 82·8             | 161              | 71·7             |
| 200              | 93·3             | 180              | 82·2             | 160              | 71·1             |
| 199              | 92·8             | 179              | 81·7             | 159              | 70·6             |
| 198              | 92·2             | 178              | 81·1             | 158              | 70·0             |
| 197              | 91·7             | 177              | 80·6             | 157              | 69·4             |
| 196              | 91·1             | 176              | 80·0             | 156              | 68·9             |
| 195              | 90·6             | 175              | 79·4             | 155              | 68·3             |
| 194              | 90·0             | 174              | 78·9             | 154              | 67·8             |
| 193              | 89·4             | 173              | 78·3             | 153              | 67·2             |

| Fahren-<br>heit. | Centi-<br>grade. | Fahren-<br>heit. | Centi-<br>grade. | Fahren-<br>heit. | Centi-<br>grade. |
|------------------|------------------|------------------|------------------|------------------|------------------|
| °                | °                | °                | °                | °                | °                |
| 152              | 66·7             | 111              | 43·9             | 70               | 21·1             |
| 151              | 66·1             | 110              | 43·3             | 69               | 20·6             |
| 150              | 65·6             | 109              | 42·8             | 68               | 20·0             |
| 149              | 65·0             | 108              | 42·2             | 67               | 19·4             |
| 148              | 64·4             | 107              | 41·7             | 66               | 18·9             |
| 147              | 63·9             | 106              | 41·1             | 65               | 18·3             |
| 146              | 63·3             | 105              | 40·6             | 64               | 17·8             |
| 145              | 62·8             | 104              | 40·0             | 63               | 17·2             |
| 144              | 62·2             | 103              | 39·4             | 62               | 16·7             |
| 143              | 61·7             | 102              | 38·9             | 61               | 16·1             |
| 142              | 61·1             | 101              | 38·3             | 60               | 15·6             |
| 141              | 60·6             | 100              | 37·8             | 59               | 15·0             |
| 140              | 60·0             | 99               | 37·2             | 58               | 14·4             |
| 139              | 59·4             | 98               | 36·7             | 57               | 13·9             |
| 138              | 58·9             | 97               | 36·1             | 56               | 13·3             |
| 137              | 58·3             | 96               | 35·6             | 55               | 12·8             |
| 136              | 57·8             | 95               | 35·0             | 54               | 12·2             |
| 135              | 57·2             | 94               | 34·4             | 53               | 11·7             |
| 134              | 56·7             | 93               | 33·9             | 52               | 11·1             |
| 133              | 56·1             | 92               | 33·3             | 51               | 10·6             |
| 132              | 55·6             | 91               | 32·8             | 50               | 10·0             |
| 131              | 55·0             | 90               | 32·2             | 49               | 9·4              |
| 130              | 54·4             | 89               | 31·7             | 48               | 8·9              |
| 129              | 53·9             | 88               | 31·1             | 47               | 8·3              |
| 128              | 53·3             | 87               | 30·6             | 46               | 7·8              |
| 127              | 52·8             | 86               | 30·0             | 45               | 7·2              |
| 126              | 52·2             | 85               | 29·4             | 44               | 6·7              |
| 125              | 51·7             | 84               | 28·9             | 43               | 6·1              |
| 124              | 51·1             | 83               | 28·3             | 42               | 5·6              |
| 123              | 50·6             | 82               | 27·8             | 41               | 5·0              |
| 122              | 50·0             | 81               | 27·2             | 40               | 4·4              |
| 121              | 49·4             | 80               | 26·7             | 39               | 3·9              |
| 120              | 48·9             | 79               | 26·1             | 38               | 3·3              |
| 119              | 48·3             | 78               | 25·6             | 37               | 2·8              |
| 118              | 47·8             | 77               | 25·0             | 36               | 2·2              |
| 117              | 47·2             | 76               | 24·4             | 35               | 1·7              |
| 116              | 46·7             | 75               | 23·9             | 34               | 1·1              |
| 115              | 46·1             | 74               | 23·3             | 33               | 0·6              |
| 114              | 45·6             | 73               | 22·8             | 32               | 0·0              |
| 113              | 45·0             | 72               | 22·2             | —                | —                |
| 112              | 44·4             | 71               | 21·7             | —                | —                |



Conversion of thermometer degrees—

°C to °R, multiply by 4 and divide by 5.

°C to °F, multiply by 1·8, then add 32.

°R to °C, multiply by 5 and divide by 4.

°R to °F, multiply by 9, divide by 4, then add 32.

°F to °R, first subtract 32, then multiply by 4, and divide by 9.

°F to °C, first subtract 32, then divide by 1·8.

**The Balance.**—This instrument is perhaps the most valuable which a chemist possesses, since, by its use, he is able to determine with a surprising degree of accuracy the exact weight of any substance.

It would be out of place here to enter upon a complete treatment of the mechanical theory of the balance, this can be obtained in special and larger works on the subject. Practically it consists of a perfectly rigid metal beam suspended near its centre of gravity on a fulcrum, the substance under comparison being suspended from pivots placed at either extremity of the beam, equidistant from and in the same horizontal line with the fulcrum in the centre. The beam usually rests, by means of a triangular piece of steel termed a knife-edge, on a plate of polished agate. At the beam ends a similar arrangement exists, the knife-edge, however, being reversed and supporting an agate plate, from which depends a hook, with wires for suspending the weighing pan.

Any inequality in the weight of the arms or pans is compensated for by means of a small metal vane fixed on the exact centre of the beam above the central knife-edge,

which, by being turned to the right or left, effects the desired correction, or by a weight projecting from one end of the beam, the precise distance of which from the centre is adjustable by means of a screw. The movements of the beam are indicated by a vertical pointer, which oscillates before a scale fixed to the pillar. To preserve and protect the instrument from the acid fumes of a laboratory, from the moisture frequently present, and from air currents, which might interfere with its action during weighing, it is enclosed in a glass case, the front or sides of which open. It is best to form a habit of always using one particular pan for the weights as a precaution against inequalities in adjustment or construction of the balance. To preserve the delicate mechanism from injury by damp, and prevent the absorption of water and consequent gain in weight by substances during the operation of weighing, it is usual to keep in the case a beaker filled with lumps of fused chloride of calcium, or one containing a little pumice stone soaked in strong sulphuric acid, both of which materials have great affinity for water, absorb all aqueous vapour, and preserve the interior of the balance in a perfectly dry condition.

Occasionally a balance requires a careful removal of its various parts, which, after being taken out, are placed on a clean sheet of paper, carefully wiped with a chamois leather, and returned to their positions. Great care is necessary in weighing that no portion of the substance be allowed to fall on to the pan itself, and in no case should materials be placed directly on the pan, but in a tared beaker, watch glass, or crucible. Should any particle inadvertently drop on to the pan, it should at once be

carefully wiped or brushed off, as not only would the accuracy of the weighing be interfered with, but frequently the surface of the pan is acted upon and corroded, to the serious damage of the balance. The greatest care is essential when using this instrument, and it is not infrequently a somewhat difficult matter for a brewer, accustomed to deal with large bulks and weights, to school himself to that attention to minutiae which is necessary to ensure satisfactory results. With care a balance will last for a number of years with unimpaired accuracy and sensitiveness, but it will only do so when attention is given to the points just enumerated.

**Weights and Weighing.**—A set of weights extending from 50 grammes to a milligramme will be found most generally useful, the larger weights being made of brass, the smaller of platinum or aluminium. These are contained in a box furnished with small forceps, by which the weights should invariably be handled, and never touched with the fingers. The smaller weights—preferably made of stout platinum foil—are turned up at one corner for holding in forceps. Instead of employing the very small milligramme weight, a wide “rider,” weighing 1 centigramme, is generally used, which is suspended over a graduated scale along the beam of balance, such scale being graduated in 10 divisions, each of which corresponds to 1 milligramme. Thus the “rider” placed on the extreme end or tenth division of scale, immediately over axis of pans, equals 1 centigramme, or, what is the same thing, 10 milligrammes; placed on the fifth division it equals but half this amount, *i.e.*,

5 milligrammes ; on the first division a tenth of this amount, 1 milligramme, and so on.

Sometimes the arm of the balance is divided into 12 divisions, in which case the "rider" weighs 12 milligrammes. Each division, of course, then corresponds to 1 milligramme.

Weights purchased from well-known makers are usually wonderfully accurate and reliable, and more than fully equal to the ordinary requirements of the student. Occasionally they may with advantage be wiped with a leather, but on no account are they to be scraped or cleaned otherwise than in the manner directed, except, perhaps, the smaller weights of platinum. These may be passed through a smokeless Bunsen flame, which will remove any organic deposit that may have become attached after prolonged use, but in no case must those of aluminium be similarly treated.

Not unfrequently, after the lapse of time, the brass weights will become slightly tarnished and discoloured, or perhaps to a very slight extent corroded ; but in the case of the former—or the latter if but very slight—no perceptible alteration will be made in the weight of the pieces.

In the operation of weighing several precautions are necessary.

The body to be weighed must never be placed upon the pans when warm, as the heat produces an upward current of air, which will cause the substance to appear lighter than is the case. This produces a greater diminution of weight than is generally supposed. The air being warmed affects also the metal of the beam at the end against which it

strikes, and thus by causing its expansion disturbs the equality in length of the two arms. In dealing, therefore, with a warm object it must, prior to weighing, be cooled, and to avoid absorption of moisture by the substance, or condensation on its surface, it is placed whilst warm in a desiccator, and when quite cold transferred to the pan of balance, and weighed as rapidly as possible. This rapid weighing is essential to secure accuracy, as there are but few bodies which do not alter in weight if the operation be protracted. Practice, and practice alone, can give the necessary manipulative dexterity for this purpose; but, in any case, should a substance which has been previously dried have gained in weight during the period occupied in obtaining such weight, it is necessary to return it to the drying apparatus and allow it to remain there for some time, weighing again until it is constant. The operation in the second and subsequent instances may be very rapid, since the weight will be known to within a small limit, and the approximately correct amount may be placed in the pan before taking the object from under the desiccator.

In weighing it is absolutely necessary to remember that under no circumstances whatever must anything be removed from the pan of the balance when the beam is either in motion or free to oscillate. The slightest neglect of this precaution will quickly ruin the instrument. Every time a weight is to be altered the beam must be thrown out of gear. No substance, as before explained, should be placed directly on the pan in order to ascertain its weight, nor should the weights, beaker, or watch glass, etc., be allowed to remain on it for any length of time, or the balance

will be injured. Again, the balance case should never be left open when not in use, and it should be closed while ascertaining the final weight by means of the rider. These precautions may appear excessive, but it is only by attention to small details that accurate and satisfactory results can ever be obtained.

**Measuring and Measures.**—The process of measurement in chemistry is practically confined to that of gases and liquids, but the latter alone will be here dealt with, for not only is the measurement of gases an operation requiring the nicest and most delicate manipulation, such as it would be difficult for an ordinary brewer to acquire, but it is almost unnecessary for the analysis of the materials with which we shall deal.

The measurement of fluids is now possible with far greater accuracy than formerly—chiefly owing to the efforts of Gay-Lussac and Mohr, and the improvements they introduced in instruments for that purpose; still, even now, it cannot claim an accuracy equal to that of direct weighing. But as the inaccuracies inherent to the process may, in most cases, be reduced to almost harmless proportions by suitable precautions, it is now resorted to very largely in analytical operations, since it is far more rapid than weighing.

There are two descriptions of measuring vessels; those which serve to measure out one definite quantity of fluid, and those by which varying quantities may be measured at will. In the former class we have measuring-flasks and pipettes graduated at one mark. The form of these is of



course well known, and it is only necessary here to refer to suitable means for testing their accuracy.

As a general rule graduated flasks, burettes, and pipettes are sufficiently correct for all practical purposes and there are very few small laboratories possessing a balance suitable for their adjustment. These vessels may, however, if it is desired, be tested in the following manner.

**Flasks.**—The flask is carefully cleaned with an abundance of tap water, afterwards rinsed with distilled water, and left inverted to drain. If not perfectly dry it may be gently heated over a small Bunsen flame, with constant movement so as to prevent its fracture. The steam and moisture are then sucked out by means of a piece of glass tubing, repeating this until the interior is perfectly dry. Care must be taken to see that on cooling no condensed vapour appears on the side of the flask, and if there is any difficulty the operation may be facilitated by the introduction of a little ether, allowing this to float over the whole inner surface of the vessel, warming gently and applying suction as before.

The flask is now placed upon a balance capable of turning with a decigramme and counterpoised.

The weight corresponding to the reputed capacity of the flask is then placed on the scale, and distilled water at a temperature of 60° F. added to the flask, until the balance is in equilibrium.

Should the level of the water in the neck of the flask correspond with the mark on the same, the graduation is correct; should it be otherwise, a fresh mark must be made by means of a diamond or sharp file, being careful not to produce too deep a scratch, or the neck will be liable to fracture at that point.

This graduation gives its contents, but although a flask may contain, say, 500 c.c., yet it will not deliver the whole of this quantity, for some will remain on the inner surface, and consequently only some 498 c.c. will be obtained. It is, therefore, usual to doubly graduate such vessels with both a contents and a delivery mark; to obtain the latter the flask is rinsed round with water, drained for a few seconds, and tared, the weight corresponding to the value of the flask being then placed on the scale, the whole counterpoised by the addition of distilled water, and the graduation made at the level of the liquid.

The lower mark on the neck then corresponds with the contents, the upper with the delivery value of the vessel.

**Pipettes Graduated at One Mark.**—These have no contents indication, that is, they are not made to *hold* any definite quantity, but merely to *deliver* a certain amount.

There frequently exists much discrepancy in measurements by means of pipettes in the hands of different experimenters, chiefly on account of the diversity of methods which are adopted for emptying the same. The best is that of allowing the liquid to run out by means of its own specific weight, placing the point of the pipette against the side of the vessel into which the liquid is to be introduced, expelling the small quantity of fluid retained in the point by blowing with the mouth.

The pipette is then allowed to remain with its lower extremity still touching the moist side of the vessel for some few seconds, during which time more liquid will drain down, this being finally emptied by again blowing.

Now this operation, simple as it seems, is capable of being performed with such variations, that, unless care be taken, great discrepancies may occur between successive measurements by different, or even the same, operators. This is not only due to dissimilar lengths of time allowed in the draining, but also to the particular angle at which the pipette is held.

The first source of error may be neutralised by uniformly allowing the pipette to drain for a definite time—say 10 seconds—and the second may be lessened by



endeavouring, as far as possible, to maintain the angle of a pipette, when draining against a beaker or flask, at about  $15^{\circ}$ .

These possible sources of error are pointed out, not for the purpose of leading to the conclusion that measurement by pipettes is so uncertain as to be worthless for purposes of strict accuracy, but rather to indicate the weak points of the process, and the precautions necessary to secure uniformity of result.

The correctness of the graduations of pipettes may be conveniently ascertained as follows :—

Carefully dry and tare a small beaker, then by means of suction fill the pipette to be tested with distilled water at  $60^{\circ}$  F. to its mark, allowing the liquid to run into the beaker and to drain for the period of time adopted with all such instruments, say 10 seconds, bringing the lower point in contact with the side of the beaker, and maintaining the angle between it and the pipette at somewhere about  $15^{\circ}$ .

The beaker should now, of course, if the pipette be correctly graduated, have an increased weight in grammes corresponding to the contents of the pipette in cubic centimetres.

If, as is usually the case, these do not exactly agree, the pipette must be re-graduated. Let us suppose that we are testing a 25 c.c. pipette; our beaker carefully tared weighs 12.535 grammes, and after delivery of the contents of pipette, observing all the precautions already mentioned, the total weight is 32.322 grammes. The water delivered weighs, therefore, 19.787 grammes instead of 20, and consequently the pipette delivers .213 gramme too little.

We now gum a narrow strip of paper upon the neck of pipette, and making a pencil mark at the point which we suppose will correspond with the required increase in quantity, again fill with water and run into re-dried beaker, repeating this until the exact point has been found from which 20 grammes is delivered. A sharp file is now taken, and placing its edge on the pencil line, a mark is filed on to the glass, the paper then washed off, and the line may, if desired, be continued completely round the neck.

**Measuring Instruments by which Varying Quantities may be Delivered at Will.**—To this form belong burettes and certain forms of pipettes.

**Mohr Burettes.**—It is to Mohr we owe the form of burette now most commonly in use, namely, that of a graduated tube drawn out at its lower extremity, over which a piece of narrow caoutchouc tubing, a few inches long, is passed, and into the other end of which a small piece of glass tubing is fixed, terminated by a finely drawn-out point at its lower extremity. There is a small space—about 1 inch—between the two tubes, and upon this is fixed a metallic clamp, which cuts off the connection between the graduated cylinder and the small glass tube. By pressing, however, the ends of the clamp, a passage is opened for the liquid, which accordingly flows out of the lower tube. By this arrangement the measurement of a liquid may be regulated with the greatest exactness. Instead of the caoutchouc arrangement a glass tap is sometimes substituted, this being more especially useful when dealing with fluids acting upon india-rubber, such as iodine, strong alkali, and permanganate of potash.

**Gay-Lussac's Burette.**—Gay-Lussac introduced a very useful form of burette, consisting of a graduated tube, closed at the lower extremity, but pierced at the side, close to its base, by a small tube at right angles to the instrument, and immediately bent upwards, so that it stands close to, and runs parallel with, the graduated tube. This tube rises to the top of the burette, where it is bent over for delivery. The burette is fixed into a wooden stand, and when used is

taken up by its foot in the left hand, and, the upper end resting upon the edge of the beaker containing the liquid to be tested, the fluid is allowed to drop from the burette, a slight elevation or depression determining its flow.

This instrument being frail and liable to breakage, has perhaps for this reason fallen somewhat into disuse. It may, however, prove useful in certain instances.

**Bink's Burette.**—Another form of burette is that known as Bink's. This is sometimes also termed the English burette, and was formerly extensively used. It consists of a graduated cylinder closed at the bottom and drawn out to a fine point at the top, this point being curved slightly downwards. Into the side of burette, near the top, is inserted a small tube, which is used both for filling the instrument and regulating the flow of its contents. When in use the thumb or finger is placed upon the orifice, and the burette inclined so that the liquid would naturally flow from the point by means of its own specific weight, the stream of fluid being regulated or arrested as required. The instrument is now placed upright, any drop remaining in the exit tube drawn into the burette by suction at the filling orifice, and the reading made.

This form of burette is not, however, so commendable as is that of Mohr, which has been previously referred to.

Pipettes belonging to this class are also employed, these being in fact nothing more or less than small burettes, the flow of the fluid being regulated by closing the upper extremity by means of a finger or thumb instead of the clamp arrangement in the former. In this case the tube is simply drawn

out to a fine point at its lower end, and also slightly contracted at the top. They are most useful for the rapid measurement of small quantities of liquid, and are not usually made to contain more than 10 c.c., but where strict accuracy is necessary, it is far better attained by the use of the burette, which enables the operator to regulate the delivery of the liquid with complete exactness.

It is, of course, easy to test the accuracy of these instruments, and this may be done in a precisely similar manner to that indicated for pipettes graduated at one mark only, but it will be seen at once that it is impossible to correct any inaccuracies existing; all that can be done is to note the amount of such, and deduct from or add them to the readings obtained.

In connection with graduated instruments, it is a point of much importance that the reading off be correct. Again we are indebted to Mohr for an ingenious little device for facilitating this object; a broad strip of black paper is pasted on a white card, and this is held behind the burette so that the edge of the black paper is about a tenth of an inch below the dark zone of the liquid. By this means the lower edge of the fluid is sharply defined, and may be read off most distinctly. In doing this, the level of the liquid must always be brought to the direct line of vision, the burette being held in that position perfectly upright, and the lower extremity of the meniscus being that noted.

**Erdmann's Float.**—A very convenient aid to exact reading off is an Erdmann's float. This is merely an elongated closed glass bulb containing a small quantity of

mercury, and having a diameter slightly less than that of the burette itself; round this bulb is etched or scratched a perfectly regular line. This bulb floats in the liquid, and the position of the line surrounding it, instead of that of the meniscus, is read off, being careful that when so doing the operator's eye is in such a position that the line on each side of bulb is exactly level, that is to say, only one line is apparent. If this is done, greater exactness of measurement is attained than by any other method.

Burettes are made of various sizes, but those most generally convenient contain 50 c.c. and 25 c.c., being divided into tenths of a cubic centimetre.

It must always be remembered that all graduations refer to liquids at 60° F., and although the instrument may be filled with the fluid of this temperature, it is necessary to exercise caution that its temperature is not materially increased during its use. In titrating with warm solutions this is sometimes difficult to avoid, since the heated air and steam arising from the liquid in the flask or beaker, when placed beneath the burette so that a portion of its contents may be added, will materially raise the temperature of that remaining in the instrument, and by its expansion will indicate the use of an incorrect quantity of liquid. This may be avoided by the following very simple contrivance. In place of the short drawn-out points attached to the indiarubber of burette, a piece of ordinary glass tubing is employed, which is bent at a right angle immediately below the indiarubber. This arm is again bent at a length of about 4 inches, downwards at a right angle, and the arm is supported in any convenient manner, preferably by a clamp attached to stand,

supporting the main body of the burette. By this means the hot air never comes in contact with the graduated portion of the tube, and the error before mentioned is thus avoided.

**Desiccation.**—The use of desiccators has been previously alluded to, but it is necessary once more to revert to the subject.

All substances, it has been seen, must be weighed when quite cold, but should they have been previously heated, they would have to be exposed for some period to the atmosphere until cool, and since all bodies in cooling attract moisture in varying degrees to their surfaces, their weight would by reason of this become augmented. This applies with still greater force to many bodies which cool but slowly and to others that are deliquescent, that is to say, readily absorb moisture.

In order to obviate this, desiccators are employed. These are forms of apparatus, by the use of which substances may be allowed to cool in perfectly dry air. They are of various kinds. Perhaps the simplest as well as the most common is that consisting of an inverted glass bell jar standing on a plate of ground glass, the rim of the jar being also ground in order that the edges may exactly coincide. The rim of the bell jar, and also that part of the plate in contact with it, are smeared over with a little grease to ensure a tight joint; and common rosin ointment or vaseline, obtainable from any chemist, answers admirably for this purpose. Under the bell glass is placed a suitable shallow porcelain or other vessel containing the desiccating agent, which



is usually either sulphuric acid or fused chloride of calcium.

These, from their great affinity for water, absorb any aqueous vapour, and maintain the atmosphere in a dry state.

Resting on the above vessel is a perforated tray of metal, porcelain, or glass, and upon this is placed the substance that requires cooling. Care must be taken that if acid be used, none is allowed to splash upon the tray ; indeed, when it is necessary to have a movable desiccator, chloride of calcium is generally preferable, though glass wool moistened with sulphuric acid answers well. In all instances, however, where the apparatus may be stationary, sulphuric acid is far preferable on account of its possessing much greater desiccating power.

A modification of this desiccator is sometimes employed whose upper bell jar is fitted with a glass tap which allows a small filter pump to be connected with it. By securing the bell jar and exhausting the interior by means of the filter pump, a considerable vacuum is produced, in which substances can be dried with rapidity, and may, if necessary, be safely left for a considerable time without fear of their absorbing moisture, as they are under these circumstances practically out of all contact with the air.

E. Fleischer (*Anal. Zeitschr.*) shows that the same substance, when dried by means of sulphuric acid, left after some 35 minutes 30 per cent. of the original moisture, after one hour 18 per cent., and after 105 minutes none at all ; whilst chloride of calcium, on the other hand, after two hours left 31 per cent., after four hours 25 per cent., and after six

hours 21 per cent. He condemns, therefore, the use of calcium chloride for desiccation.

**Air Bath.**—When a body requires a high temperature to drive off its moisture (provided it may be exposed to such temperature without danger of change), it may be placed in an air bath.

This is a simple form of apparatus, being merely a box of sheet copper, having a closely-fitting door in its front ; it contains a perforated shelf, and is placed on an iron tripod and heated by means of a small flame.

The student will, however, find the boiling water bath presently to be mentioned of more value than the air bath, both on account of the ease with which a uniform temperature may be maintained, and by reason of the fact that the boiling point of water is the temperature most suited to the majority of drying operations, since such substances as malt, sugar, etc., would be decomposed at the greater heat attained by means of the air bath. For driving off the water of crystallisation from salts—such as in a water residue—an air bath is necessary.

**Evaporation.**—Liquids are most commonly concentrated by direct evaporation in a suitable dish of porcelain or platinum, or in a beaker or flask, but in the case of the evaporation of water, beer, wort, etc., direct application of a flame is inadvisable, since, should the liquid boil, loss by spurting is almost unavoidable, whilst the application of a direct flame to the bottom of a vessel containing beer or wort would be liable also to burn or caramelise its contents, and for such purposes the student will generally



find that evaporation may be most satisfactorily performed by means of a water bath.

**Boiling Water Bath.**—Substances which do not undergo change at a temperature of about boiling point are usually dried, when it is desired to determine their moisture, in a boiling water bath.

A useful piece of apparatus is that of a combined evaporating bath and drying oven. Various forms of these baths are made, but one which is both simple in construction, and meets most of the requirements of a brewer's laboratory, is simply a square or round vessel made of sheet copper, partially filled with water, and heated by means of a Bunsen flame.

It is fitted with an oven of about 6 inches square, and closed by means of a door. In the top of the water bath are two evaporating holes of about 3 inches in diameter, fitted with rings of various sizes, so as to enable the diameter of the holes to be reduced if required. A large hole of about  $4\frac{1}{2}$  inches diameter is also provided for heating a flask of distilled water, as well as two funnel jackets for drying precipitates, etc. Covers should be provided for the holes, so that when not in use these may be shut down.

The water in the bath is from time to time replenished, and it is an advantage to have an arrangement attached for a continuous supply of water to replace that converted into steam.

The following is the description of one which may be adopted. Into one side of the bath, about a third of the

distance up, a small tube some 2 inches long is inserted, attached to which is a piece of ordinary brass pipe having a diameter of about 2 inches and a length of 4 inches, the pipe being open at both ends and standing in a vertical position. The bottom is closed by means of a tightly-fitting indiarubber cork, through which passes a piece of ordinary glass tubing, constituting the overflow or waste pipe. Into the top of the brass tube mentioned a continuous supply of water is allowed to slowly flow by means of piping connected with the water tap, and the level of the liquid inside both the outer receiver and the bath itself is regulated as desired, by sliding up or down the glass tube passing through the indiarubber cork.

After the evaporation of any liquid in a water bath, it is usual to complete the drying operation by placing it for a short time in a steam bath oven, prior to finally weighing it, and in evaporating liquids it must be remembered that both glass and porcelain are attacked by many solutions, particularly if alkaline, and thus error—sometimes serious—may be introduced into analyses. The estimation of alkalies may be affected by this cause.

**Filtration.**—Filtration is one of the most important processes connected with quantitative analysis, since when determining any substance gravimetrically the operator seeks to bring it into some insoluble form, and to estimate the amount of this by washing, drying, and weighing it. The separation of a precipitate from the liquid in which it is formed may be accomplished by decantation, filtration, or a combination of both.

In general it is advisable to allow a liquid to stand at rest from some time after the addition of the precipitant, for not only does more complete separation of a substance in an insoluble form occur, but if thrown immediately upon a filter many precipitates are apt to pass through the pores of the paper and yield a cloudy filtrate. Before commencing to filter, the operator should always ascertain that there is sufficient of the precipitant present by adding a further small quantity of it, carefully noting if any cloud is produced, and this may of course be ascertained with far more certainty after the precipitate has settled.

**Decantation.**—Decantation is not very frequently resorted to, for the operation is lengthy, and the amount of washing water necessary is large. A few very heavy precipitates may, however, be treated in this way, but it must be those only which are practically insoluble in water. It may be pointed out that no precipitates are absolutely insoluble; but in many cases their solubility is so small that practically large quantities of water may be used for washing without perceptibly affecting the results. Filtration is most conveniently effected in glass funnels, the sides of which should be perfectly regular and inclined at an angle of  $60^\circ$ , the stems narrow and long, and cut off at the end obliquely; those having a diameter of 3 inches are about the most convenient for general use. The filter paper should be carefully folded into four, placed in the funnel, moistened with hot water, and arranged so as to fit closely to the glass.

In most operations hot distilled water is used for washing, liquids filtering much faster when warm. There are cases, however, when its

use is inadmissible, weak ammonium hydrate or other liquids having to be substituted. These instances will be referred to in their proper place. When it is necessary to avoid alteration in the bulk of liquid by dilution, a filter must not be moistened at all prior to filtration.

A badly-fitting filter paper is the frequent cause of much annoyance, both by filtering slowly and becoming easily ruptured.

The stem of the funnel should be arranged so that it touches the side of the beaker or other vessel into which the filtrate is to be received; by this means splashing is avoided, and, by capillary attraction, speed of flow is accelerated. In filtering, it is generally advisable to first run the supernatant and comparatively clear liquid through the paper, as by this means its pores swell, and it becomes more able to perfectly retain the precipitate. Having done this, the precipitate is washed on to the filter by means of boiling distilled water, using either a glass rod with a small tip of narrow vulcanite tubing, or a feather, to detach any precipitate not otherwise readily removed. The article used for this must, of course, be in its turn carefully rinsed, and the liquid during filtration should never be poured directly into the funnel, but down a thin glass rod, this being held so that the liquid falls against the side of the filter. If the liquid be poured into the apex, loss by splashing will certainly result, together with great danger of rupturing the paper itself.

Now, having transferred the whole of the precipitate from the vessel into the filter, and having most carefully seen that not a particle remains in such vessel, we proceed to wash it.

**Wash-Bottle.**—This is accomplished by means of a wash-bottle—a piece of apparatus so well known that it need not be here described. From it is directed a fine stream of water against the upper edge of the filter paper, carefully moderating the force of the jet so as to avoid rupture of the paper. When employing hot liquids, a coil of string should be wound round the neck of the flask used, in order that it may be handled the more conveniently. Instead of this a stout piece of india-rubber tubing may be substituted, having a diameter of, say, 1 inch, which is either 'stretched over the neck or slit down the side, so that it may be easily removed from one flask to another, its natural elasticity being sufficient to prevent its dropping off. If a stream of water be allowed to flow directly on the lower portion of the filter, one of two misfortunes is almost sure to happen, either a portion of the precipitate will be projected out of the funnel, or the apex of the filter will give way, and thus spoil the experiment.

The operator should never re-fill the filter with liquid until the quantity previously added has run through.

A precipitate usually requires at least three thorough washings after the whole of it has been collected in the funnel, and the liquid by which this is accomplished drained through. Particular care should be taken to allow the whole of the fluid to drain through between each washing. This point cannot be too strongly accentuated, since it is one that is very frequently neglected, and inattention to it causes many unsatisfactory and worthless results.

A moment's consideration will at once render its importance evident; for if washed twice with a given quantity

of water, it is perfectly obvious that the impurity is reduced far more than by one washing with double the quantity. In any case, the last few drops of the washings should be tested either by adding a small quantity of a reagent that would indicate the presence of the minutest trace of the precipitant (such as the addition of a drop of dilute sulphuric acid to the last portion of filtrate from the determination of sulphuric acid by baric chloride), or we may evaporate a single drop on a piece of platinum foil or thin glass, observing whether there be any perceptible residuum left, in which case the washing must be further continued.

Bunsen has published some most valuable investigations upon the smallest volume of wash water and minimum number of washings required to reduce a precipitate to a given state of purity, to which the student may be referred. (*Ann. der Chim. u. Pharm.*, vol. cxlviii, p. 269.)

In certain cases, when dealing with precipitates of a very gelatinous nature, it is convenient to accelerate the somewhat tedious process by applying suction to the bottom of the funnel.

A simple arrangement for this purpose may be constructed as follows:—A stout flask is fitted with an india-rubber cork which is pierced with two holes; through one hole the stem of funnel passes, and through the other a piece of glass tubing projecting only some  $\frac{1}{2}$  inch below the bottom of the cork, and bent above it at an acute angle. To this is attached a piece of small vulcanite tubing, terminating in a mouth-piece, and provided with a metal clamp, the whole being perfectly tight.



When in use, suction is applied by the mouth to the flask, and a partial vacuum produced therein, the clamp being open at time of suction, and immediately afterwards closed. By the occasional repetition of this, filtration is much accelerated, and the most gelatinous precipitates may be washed with comparative ease.

A word of caution, however, must be added, for if the suction is not applied gently there is danger of rupturing the filter ; but this may to a certain extent be prevented by placing a small platinum cone at the bottom of funnel, thus protecting the apex of filter.

**Filter Pump.**—There are several forms of apparatus by which a partial vacuum may be produced, one of the simplest, and at the same time most efficient, being that of a Bunsen pump. In this, the weight of a column of water is used as a suctional agent, and a constant vacuum, the amount of which may be regulated at will, is thus obtained. It forms a most valuable adjunct to the ordinary apparatus of a laboratory.

**Ignition of Precipitates.**—Having now completed the filtration of a precipitate, it is in most instances necessary to dry and ignite it. The former is very easily effected by means of the steam bath, the precipitate being placed inside the chamber, or, still better, in a funnel jacket specially constructed for this purpose and attached to the same. It may also be dried in the following manner:—Support the funnel by means of a triangle, on the top of a simple cylinder of tin or iron of about 14 inches in length, and 5 inches diameter, and stand the whole on a tripod

furnished with a wire gauze, underneath which a small flame should be placed. The method first mentioned is, however, in every respect preferable. Some precipitates are weighed in this state, *i.e.*, dried at 210° F., and in that case the filter paper must have been previously dried at the same temperature, and carefully tared, preferably in a small and light glass weighing-tube provided with a closely-fitting stopper.

The precipitate and filter, when apparently perfectly dry, are removed from bath, placed in this tube under a desiccator, and allowed to cool, the drying and weighing being repeated until constant.

The majority of the precipitates, however, are ignited before weighing, and this is accomplished in the following manner:—A platinum or porcelain crucible is heated, cooled under desiccator, and weighed; it is then placed on a sheet of glass or highly-glazed paper, about 12 inches square, together with a piece of platinum wire, and either a feather or a small camel's hair brush. The filter paper is now opened out, and the precipitate transferred, so far as is possible, into the crucible, the paper itself folded up and bound round with one end of the platinum wire. A small Bunsen burner—the flame of which must be smokeless—is now applied to the paper, which is held over the crucible in order that any portion becoming detached during the ignition may fall into it. This is continued until it is converted into a perfectly white or greyish mass, when it is carefully shaken into the crucible, which is then ignited, at first gently, and afterwards more strongly. Any particles which may have become detached will be collected on the



glass plate or paper and are transferred into the crucible, any particles adhering being removed by the feather or camel's hair brush. The heat is continued until the precipitate is white (in a few cases it will be grey, and in some black). The lid is then placed on, and the whole conveyed to the desiccator, there allowed to cool, and weighed.

With many precipitates it is unnecessary that they should be first separated from the filter paper, in which case the whole may be transferred to the crucible and at once ignited, the crucible being placed at a slight angle in order the more readily to oxidise the carbon by contact with air. It is a common error of students to burn off their precipitates by the aid of a large Bunsen flame, hoping thereby to accelerate the ignition, but this is the exact opposite of what really happens; for, when using a large flame the crucible becomes completely surrounded with the products of combustion, air is allowed no access, and consequently the carbon is oxidised but slowly. It will be found in every way preferable to employ a burner of distinctly small size, arranging this so that the top of its blue cone approaches to within an eighth of an inch of the bottom of the crucible.

Experience has shown that precipitates maltreated in the manner described may be ignited for several hours and yet not be completely burnt off, whilst the platinum crucible, if such were used, would be considerably damaged by this prolonged exposure to heat. On the other hand, ignition, when conducted as recommended above, may be completed in 10 or 15 minutes—often less—without the slightest trouble.

A fruitful cause of the difficulty frequently experienced by beginners in satisfactorily burning off precipitates is to be found in the want of sufficient care in the prior operation of washing, this often being hasty, insufficient, and improperly conducted.

**Filter Paper Ash.**—After burning off the precipitate as already described, it is necessary to deduct the ash of filter paper from the gross weight; for this reason filters should be as free as possible from inorganic matters. In order to determine this deduction, five or six filter papers should be burnt off, and the average amount of ash ascertained. Different makes of paper vary considerably in their ash percentages, the Swedish and Rhenish being the best, containing an exceedingly small amount of saline matter. The ash to be deducted for filter will be less when the liquid filtered is acid; for instance, in deducting from precipitate of baric sulphate—the filtrate of which is acid—1 milligramme would probably be correct; whilst for lime, the filtrate of which is alkaline, the same papers would give an ash of 2 milligrammes.

For some purposes, however, even a minute quantity may be objectionable, and in such cases the greater portion of inorganic matter may be removed by treatment with dilute hydrochloric acid. Several packets of filters are placed in a beaker, covered with a mixture of one part acid to twenty of water, and allowed to remain for five or six hours. At the end of this time the acid liquor is drawn off, and repeatedly replaced by distilled water until every trace of acid has disappeared. After this they are dried in a steam

bath. Such papers are extremely pure, and yield on ignition a mere trace of ash.

Filters may now be obtained ready prepared by treatment first with hydrochloric, and afterwards with hydrofluoric acid, and the ash of these is so small that no correction is necessary for them even in the most rigid and exact analysis.

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## CHAPTER II.

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### Part I.—NITROGENOUS BODIES OR PROTEÏNS.

THE student, having now acquired from Chapter I some general ideas as to the apparatus and methods with which he will have to deal in the course of his analytical determinations, could now proceed directly with malt analyses, but before doing so it is of great importance, in order that he should understand the meaning of the separate determinations made, appreciate the interpretations, and make these of real value in his practical working, for him to obtain some knowledge of the three groups of chemical substances which enter into the complex changes of barley germination, mash-tun conversion, and yeast fermentation, which three processes constitute the basis of all brewing operations.

The first group which he will have to consider are the nitrogenous bodies, or albuminoids, now grouped under the generic name of "proteins," and these substances are dealt with in Part I of the present chapter.

Parts II and III will deal with the "enzymes" and "carbohydrates" respectively—the former nitrogenous bodies of the utmost scientific interest and importance, and concerned with the breaking down of many of the substances dealt with in Parts I and III.

It is not possible to describe these three groups of sub-

stances in any detail, and they are here treated quite simply and only so far as they are of direct importance in brewing operations. Reference should be made to the various original papers and literature dealing with these important branches of brewing chemistry.

**General Properties.**—Proteïns are substances of an organic character which contain nitrogen, carbon, oxygen, hydrogen, in nearly all cases sulphur, and sometimes phosphorus. They resemble white of egg in their composition, which substance is a typical example of an albuminoid.

Our knowledge of the absolute composition of these bodies is very imperfect, but it is known that their molecule is very large and complex, and that when broken down they yield a great number of products, the exact chemical nature of many of which is not yet well understood, although it has only been by a careful study of these simpler protein compounds as obtained by the hydrolysis of the more complex bodies that it has been possible to obtain any definite ideas as to their composition and properties.

There is very great difficulty in obtaining them in a pure state, and this is accentuated by the fact that the great bulk of these proteins have neither the distinctive character nor the individual properties that would allow of their being distinguished from one another, and which are found in the great majority of chemical substances, both organic and inorganic.

Generally speaking, they are non-crystalline bodies without taste or smell, and are of course naturally prone to change or decompose, due to this complexity of composition.

It was supposed for many years that the protein bodies of vegetable origin were of the same order as those obtained from animal tissues, and that they were in many cases identical in composition and properties, but later research work, particularly that of Osborne,\* suggests that at any rate many of these proteins differ from one another, according to which of these two sources they are derived from.

One of the first proteins discovered was the substance known as gluten, and as long ago as 1819 wheat gluten was isolated by Tawei, and later separated by Ritthausen into three distinct protein bodies known as vegetable glue, mucedin, and gluten fibrine. Still more recently Osborne isolated only two, one of which was closely akin to mucedin, and which he named *hordein*, an important substance which will be again referred to presently. Gluten was obtained by mixing wheat flour with water, thereby washing away the starch and leaving gluten behind as a gummy, elastic, and buoyant mass.

It was originally thought to be present in most cereals, but is certainly only characteristically present in wheat. When flour is washed in this way not only is the gluten group separated but in the same way several protein bodies pass away in the liquid, some of which are soluble, and can be coagulated or rendered insoluble by heat, while others remain soluble even in boiling water. Others, again, are insoluble, but may to some extent be rendered soluble by enzyme activity during malting, and to some extent also during the mashing process and by yeast in fermentation.

\* *The Vegetable Proteins*, T. B. Osborne, 1909.

**Tests for Proteïns.**—Before dealing with the various classes into which these proteïn bodies can be divided, it may perhaps be desirable to give some general tests which can be used for their identification.

Solutions of the proteïns readily absorb oxygen when exposed to the air, and many of them thus become cloudy, and undergo change in constitution.

On boiling, their solutions are in some, but not all, cases coagulated, forming flocculent masses insoluble in water, alcohol, or ether.

Iodine colours them yellow, sulphuric acid produces a red or violet tint.

When nitric acid is added to a solution of proteïn, a white precipitate forms, which becomes yellow on heating. If the precipitate is filtered off and treated with ammonia, it becomes orange coloured.

A delicate test is that with a solution known as *Millon's* reagent, which is obtained by dissolving mercury in an equal weight of nitric acid, and diluting with twice its bulk of water. With this test, warm solutions of proteïns give a red coloration, or, if a considerable quantity is present, a precipitate of the same colour.

The *Biuret* reaction is a valuable one, for it not merely enables us to detect the presence of nitrogenous bodies, but, to some extent, to divide them into groups. The test consists of the addition of a drop or two of a dilute solution of sulphate of copper to the solution to be examined, afterwards adding a few drops of a strong solution of caustic soda, when a coloration is produced, reddish violet with proteoses, and red with peptones.



All proteins give off on ignition an odour resembling burning hair or feathers.

**Protein Separation.**—Very little is known of the influence of nitrogenous bodies on brewing; some are believed to be harmful, others are certainly necessary and beneficial. This lack of information arises largely from the fact that we have no satisfactory methods of separating and estimating the different varieties which we know to exist, and, until we have means of so doing, we cannot hope to make much progress. We know, however, that the mere total amount of nitrogenous matter in a malt or wort gives us no information of value; *i.e.*, we cannot say a malt or wort is good or bad just because it contains a large or small amount of nitrogenous matters. Ullick proposed a method for the separation of the nitrogenous matters of wort into groups, and some years ago the writer applied this method to the analysis of worts prepared under varying conditions, but further experience of the process convinced him of its unreliability.

Considerable work, however, has been carried out in recent years by separating out protein bodies or groups of bodies from the seeds of plants, but only those protein substances that are found in the barley seed need be mentioned.

The analysis of such a seed for the initial separating of these bodies is based upon the following:—

1. Proteins soluble in neutral or saline solution.
2. Proteins insoluble in both of these, but soluble in dilute solutions of acids or alkalies.



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3. Proteïns soluble in alcohol (70 to 90 per cent. strength).

Perhaps the most important of these groups are the alcohol-soluble proteïns, to which Osborne has given the name of "*protamines*," the particular protamine of barley being the substance already referred to under the name of *hordein*. This proteïn appears to be chiefly concerned in the enzyme changes in malt which lead to the production of the important non-coagulable nitrogenous bodies, and represents about 40 per cent. of the total nitrogen of the barley.

The total percentage of nitrogen found in barley varies considerably, and may be from 8 to 14 per cent., expressed as proteïn, the average being 10–12 per cent., whilst approximately four-fifths of this amount is insoluble in water, consisting of the glutinous bodies above mentioned. It is still a matter of some controversy whether a barley of high total nitrogen-content results, other things being equal, in a malt containing a high percentage of soluble nitrogen, though the general consensus of opinion is that it does do so, but much depends upon the particular conditions of malting.

There is no definite relation between such nitrogen in a barley and the subsequent diastatic power it is likely to produce in the malt, but it has been found from experience that with the same class of barley grown under similar soil conditions and in the same locality, the diastatic activity will vary from season to season relatively with the nitrogen-content of the barley, that is, the higher the

nitrogen the greater the tendency to increased diastatic activity.

### **Distribution of Proteins in the Barley Corn.—**

Not only are there many protein bodies in the barley grain, differing from each other in composition and properties, but there is also a very unequal distribution of these substances in the comparatively small volume of the seed. Why this should be so, can perhaps be better understood if it is realised that the seed is the storage place of the elaborated and highly complex types of protein bodies which have resulted from the growth of the plant, and, further, that such a seed also contains all the intermediate forms down to the lowest and simplest type, the whole being the result of the building up and breaking down processes which are characteristic of all living organisms. When germination commences the barley corn is at once the seat of very involved physiological changes in which the protein bodies take an active part, they being broken down into simpler and diffusible forms by enzyme action, and distributed to those parts of the seed in which active growth is proceeding, providing nourishment for the various organs, together with liberation of heat energy.

As might be expected, there is relatively a much greater supply of nitrogenous material resident in the germ of the seed, than in the very much larger volume of endosperm tissue which surrounds it. In the endosperm are protein bodies of different composition and properties, the distribution of these not being uniform throughout the seed.

J. B. Osborne has vigorously attacked the problem of their separation and identification, and the various bodies which he has successfully isolated may be found in his published reports, and in a recent monograph on the Vegetable Proteïns.

Little or nothing, however, is yet known of these substances in their relation to the brewing processes, and bearing in mind that the insoluble proteïns of barley—about four-fifths of the total nitrogen content—are, in part, transformed during malting, and in a lesser degree in mashing, to soluble forms by proteolytic enzyme action, we may more usefully proceed to examine generally the nitrogen content as it is found in the germinated barley or malt.

**Malt Proteïns.**—These may, for our present purpose, be separated generally into three classes :—

- (a) Proteïns insoluble in both hot and cold water.
- (b) Proteïns soluble in water, but coagulable and rendered insoluble on boiling.
- (c) Proteïns which remain soluble on boiling.

(a) *Insoluble Proteïns.*—But for the consideration that a portion of (a) may undergo proteolytic change in the mash tun, this group of proteïns remains undissolved in the extracted grains left behind in the mash tun, and is of no further interest to the brewer.

(b) *Coagulable Proteïns.*—The proteïns of (b), usually known as the “coagulable albuminoids,” are a group of bodies which differ from one another chiefly in the temperatures at which they coagulate or separate out

as insoluble, and would appear to be of small importance to the brewer, as during the boiling of the wort in the copper they are presumably coagulated and rendered insoluble, but practically it is of the utmost importance that complete coagulation and separation of these bodies should be obtained, and this will depend, to some extent, upon the nature of the boil and the amount and quality of the hop used, but perhaps more than anything else upon the character of these coagulable proteins, as found in the malt used. Unfortunately, our present knowledge does not enable us to determine, by analysis, to what extent harmful coagulable albuminoids may be present in a malt, but there is no question that some of the lower grade barleys and unsatisfactory malting conditions are liable to produce an excess of certain of these bodies, which will not be found in well made malt or malt made from a good barley.

In practice, thorough coagulation by aërating the hot wort is essential, together with as complete deposition as possible of these substances, which would otherwise be carried forward to the fermenting vessel, where the satisfactory conditions of yeast working and fermentation might easily be interfered with. Such proceeding is undoubtedly some sort of safeguard, but the type of proteins coagulated on boiling, or thrown out of solution on cooling, must not be forgotten when the choice of different qualities of malt is under consideration, for it is quite certain that if an excess of these objectionable bodies is allowed to get through to the finished beer difficulties will arise, and the beer may prove stubborn in brightening or fining, or prove unsatisfactory in other directions.

(c) *Non-coagulable Proteins*.—Many methods have been proposed for the separation of these soluble nitrogenous bodies, mostly depending upon the property these substances have of differing one from another in their solubility in certain salts, and the capacity which certain salts possess in saturated solution of precipitating out different groups, enabling these to be differentiated, and their properties examined ("salting out"). Such substances as phosphomolybdic acid, phosphotungstic acid, and a process largely used by Horace Brown\* in his researches upon these soluble proteins, depending upon the action of nitrous acid, have given good results with methods of precipitation.

The group of substances so obtained from a boiled cold extract of malt are divided up as follows :—

|  |     |     |     |       |
|--|-----|-----|-----|-------|
| Ammonia nitrogen...                          | ... | ... | ... | 3·5   |
| Malt-albumose nitrogen                       | ... | ... | ... | 20·0  |
| Malt-peptone nitrogen                        | ... | ... | ... | 31·0  |
| Amide and amino nitrogen                     | ... | ... | ... | 8·5   |
| Nitrogen due to organic bases                | ... | ... | ... | 4·0   |
| Balance of nitrogen still unaccounted for... |     |     |     | 33·0  |
|  |     |     |     | 100·0 |

The malt albumose and malt peptone, which constitute half the total percentage of these soluble non-coagulable proteins, are perhaps the most important, as they represent the nitrogen found to be largely assimilable by yeast, and

\* See a most important paper on this subject, *Journ. Inst. Brew.*, 1907, by Horace Brown, pp. 394–457, "The Nitrogen Question in Brewing," Part I.

presumably also by other organisms such as the bacteria. They appear to be the nitrogenous bodies mostly obtained by degradation of the hordein of barley by enzyme activity\* during germination, and stand mid-way between the original protein and the final and lowest forms.

The **Albumoses** may be separated into three different bodies, one of which has the distinctive property of producing a permanent foam, whilst all of them are neutral and, of course, soluble in water.

The **Peptones** have been separated into bodies (1) and (2), and are an intensely interesting class, having a direct power of nourishing yeast. They are produced during malting, and also, to some extent, during the standing of the mash, provided the temperature is sufficiently low. It may be said generally that the greater the amount of peptone material present in a fermentation the more vigorous will be the yeast, and the greater will be its reproduction. This is one reason why it is always found that a yeast will reach its maximum vigour when grown in a wort produced from a low-mashed malt. In any case we certainly increase the amount of nitrogenous matter in the wort when we mash at a low temperature.

Whilst peptones undoubtedly increase reproduction of yeast they do not at the same time necessarily increase the fermentative energy of the cells, but small quantities of peptone by increasing the vigour of the yeast vegetatively may, however, in this way and under certain circumstances, also increase the fermentative energy and so bring the attenuation of a beer to a lower limit.

\* "Proteolytic Enzymes," see p. 74.



So far as is known excess of peptone bodies in the wort do not in themselves necessarily tend to increase instability in the finished beer, for although the assimilable nitrogen content of the wort is higher than would otherwise be the case, yet it appears practically that, within certain limits, so long as there is peptone present in a wort under normal conditions yeast cells will go on reproducing until the whole of the peptone is assimilated. Thus it is a well-known fact that one of the most powerful preventives of bacterial growth and contamination of a beer is the vigour of the culture yeast, and if the yeast cells remaining in the beer are healthy and energetic there is less danger from bacteria or even so-called wild yeast cells as opposed to culture types than would otherwise be the case, although H. Brown is of opinion that an excess of peptone-like bodies is probably responsible for the after-growth of wild yeasts and bacteria. Peptones are white amorphous substances, soluble in water and non-coagulable by heat. They are precipitated by tannic acid, alcohol and certain substances, chief of which is phosphotungstic acid. Mineral acids in small quantities do not precipitate them.

**Amides and Amido-acids.**—These are supposed to be the end products of the hydrolytic action of peptase upon the proteins, and probably they are also produced in other ways in the plant for the elaboration of the more complex proteins. It is probable that they have not nearly so great a nutritive value for yeast as have peptones. They are crystalline substances, and the best known of the group is asparagine, so called because it was first separated from the sprouts of the asparagus plant.

Asparagine is present in the rootlets of malt, but is probably not present in malt itself.

Amido-acids are formed when amides are heated with a mineral acid. Practically nothing is known of their brewing value.

### Assimilable and Permanently Soluble Nitrogen.

—In separating the assimilable nitrogen from the permanently soluble nitrogen H. Brown made use of yeast in a series of fermentations carried out under the optimum conditions for the assimilation of nitrogen by the yeast-cell. In this way he found that 42 per cent. of the total soluble nitrogen could be taken up and assimilated by the yeast. The amount, however, taken up by the yeast during an average fermentation, as under brewery conditions, varied from 20 per cent. to 35 per cent. The difference between these latter percentages so obtained and the full value of 42 per cent. represents in any particular case the residue of assimilable nitrogen which will be found ultimately in the finished beer, and this amount will vary in its greater or less quantity with the conditions of fermentation and other influencing factors. These experiments indicate that the greater or less amount of this assimilable nitrogen so left in the beer is of great importance for the history of the beer subsequent to the racking stage.

What practical value or direct influence on the after conditions of brewing the undifferentiated soluble nitrogen classed as the *permanently* soluble may have in distinction to the *assimilable* portion is at present unknown.

Finally some important conclusions obtained by Horace Brown as resulting from the experiments detailed in this



paper (which should be most carefully studied)\* are as follows :—

The influence of the temperature of mash on the extraction of the permanently soluble and assimilable nitrogen of malt is very marked, and the permanently soluble nitrogen extracted under standard conditions increases slowly, but almost uniformly, with the temperature between 60° F. and 100° F. This latter temperature coincides with a critical point beyond which the extracted nitrogen increases rapidly up to 110° F. From this point the curve expressing the nitrogen values begins to flatten sensibly, and attains its highest point at 120° F., which marks the point at which the peptonising and proteolysing enzymes are exerting their maximum effect on the otherwise insoluble proteins.

At 120° F. the permanently soluble nitrogen extracted amounts in the case of an English malt to about 40 per cent. of the total nitrogen of the malt, 23 per cent., or thereabouts, being derived from the "ready formed soluble nitrogen" of the malt, and 17 per cent. from the products of proteolysis.

Between 120° F. and 140° F., the extraction of permanently soluble nitrogen decreases somewhat, indicating that we are now in a region of temperature that is beginning to restrict the action of the proteolysing enzymes. This effect becomes still more marked between 140° F. and 150° F. This latter temperature is that of the ordinary infusion mash, and at this point a degree or two either way makes a considerable difference in the nitrogen extracted. The ordinary mashing temperature, in fact, marks a critical point for the

\* "The Nitrogen Question," Part II, *Journ. Inst. Brew.*, 1909, pp. 169-296, by Horace T. Brown.

proteolysing enzymes, just as it is well known to do for the diastatic enzyme.

From about 155° F. the extraction of permanently soluble nitrogen further diminishes until 180° F. is reached, when the amount corresponds with that extracted at 60° F., and remains constant until the temperature of boiling water is reached. The temperature of 180° F. or thereabouts marks the stage at which the proteolysing enzymes are rendered inactive.

## Part II.—ENZYMES.

Effront has remarked that the study of brewing is responsible for two great discoveries—Pasteur experimenting with beer yeasts produced sufficient evidence to finally dispose of the theory then generally held of spontaneous generation, and Dubrunfaut working with malt laid the foundation of the science of enzymes and their action; and the more we come to understand of the ultimate nature of enzyme activity, the nearer we shall be to obtaining some clear ideas as to the complex and wonderful changes that culminate in the vital activity of the living organism.

The term soluble ferment appears at first a somewhat confusing one, since we usually associate the expression "ferment" with certain organised microscopic bodies such as yeast or bacteria, whose function it is to split up complex substances, principally members of the carbohydrate group, into various gaseous and other products. This confusion of terms is due to the replacement of Liebig's theory of fermentation by that of Pasteur. The former, it will be remembered, argued that fermentation was caused by the mere contact of

albuminous matters in a state of decomposition with carbohydrate matter—that is to say, that nitrogenous bodies in a state of chemical change, when undergoing what was termed molecular disturbance, induced a similar change in bodies of the carbohydrate type. But when Pasteur demonstrated that fermentation was produced by organised cell life, it cast an altogether different light upon the meaning of the word ferment. To distinguish the two classes, they were accordingly divided into organised (zymes) and unorganised, or soluble, ferments (enzymes).

The still more recent discovery, however, by Buchner,\* that the enzyme, or, perhaps more accurately, enzymes, of fermentation, producing carbonic acid and alcohol from sugar, could be isolated from the living yeast cell, and are able in the free state, apart from the living plant, to produce active decomposition of sugar, has advanced the science of enzyme action a step further than where Pasteur left it. As a result, it is evident that all enzymes may now be classed as unorganised or soluble ferments. In other words, living protoplasm, whilst it may be essential for the *production* of enzymes, is not essential for their separate life and activity.

It is singular to observe that, so far as our present knowledge extends, enzymes undergo no change in their composition during their action, yet they are capable of performing only a definite, although very large, quantity of work; that is, although they undergo no apparent alteration in substance, their energy becomes after a time exhausted, and all action ceases.

But this appears to be—at any rate, partly—due to the

\* *Die Zymase Gärung*, 1903.

decomposition or excretory products which the enzymes themselves produce, and it is found in many cases that where the products of decomposition are in part or wholly removed, the energy of the enzyme is revived, and renewed activity takes place. A practical example of this is found in the fact that at a stage of the mashing process enzyme activity will more or less completely come to an end, but with dilution of the mash it is again renewed, thus "under-letting" will serve under some conditions to produce increased conversion power and a correspondingly greater production of the more readily fermentable maltose.

**Nature of Enzymes.**—Enzymes and proteins generally belong to a class of substances known as colloids, bodies in which a very large surface condensation can take place, resulting in a surface combination by "adsorption," characteristic of colloids in general. This peculiar action occurs wherever there is contact between the surface of gases and liquids; and another example of this condition is seen when filter paper or other fibrous material is dipped into a solution of colouring matter, such as, for example, Congo red, when, if the dye is of just sufficient strength, the filter paper will take up the whole of the colouring matter and leave a colourless solution.

Again, there appears to be some analogy between enzyme action and that known as catalytic, first suggested by Berzelius, and the theory has been considerably developed in recent years.\*

Catalysts are bodies which alter the rate of any chemical reaction, generally by accelerating, very rarely by retarding,

\* *The Nature of Enzyme Action*, by W. M. Bayliss, D.Sc., F.R.S.

and, as a general rule, it is found that their peculiar properties are very similar to those of enzymes.

There are a number of examples of catalytic action both in inorganic and also organic chemistry, and in many of these reactions the catalyst is known to form definite chemical combinations, but only temporarily and only in such a way as to bridge over or facilitate the reaction taking place between the main substances concerned.

Thus the conversion of alcohol into ether by means of sulphuric acid is an instance in which an intermediary substance ethyl sulphate acts as the catalyst, it being split up again immediately it is formed.

Another example of a different order is afforded by the inversion or hydrolysis of cane sugar in the presence of a trace of acid, the acid in this case acting as the catalyst.

Generally speaking catalytic action and enzyme activity are similar in the following respects :—

(1) Both catalysts and enzymes remain unchanged at the end of any reaction in which they are engaged.

(2) There is no increase in the weight of either substance after the reaction is finished.

(3) Neither have the power of starting a chemical reaction, and finally

(4) There is no resulting chemical combination with the final products of the reaction.

Nägeli has formulated an entirely different theory of the phenomena of enzyme action, and he explains enzymes from the physical point of view as quite distinct from the chemical, their action being considered as a property and not a substance, and their activity as being caused by a kind of resonant vibration of waves set up in the

substance which vibrate sympathetically with waves in the enzyme molecule. This, it will be remembered, is very similar to Liebig's old theory of molecular wave movement as the cause of fermentation, and on the whole enzymes may best be regarded as colloidal catalysts, as this theory perhaps better than any other explains the phenomena of their action.

Enzymes are essential to the life of the organism producing them, due to their power of breaking down complex substances into simpler forms, and by this means supplying the vegetable or animal organism with soluble and diffusible food supply, together with the necessary heat required for its growth.

They are, in fact, potential heat producers, and considerable quantities of heat are often obtained in any reaction in which they are engaged. Thus it has been found that one molecule of glucose fermented into alcohol and carbonic acid gas will set free 71 heat units or calories.

The simpler bodies resulting from this decomposition or splitting up of the complex substance may also be thrown off as excretory products of no known further use to the plant, as, for example, carbonic acid and alcohol in sugar decomposition, or they may produce substances only partially broken down, which after being assimilated by the plant can at a later stage be built up again into more complex forms. This is due to a peculiar reversibility of action whereby enzymes not only break down insoluble substances into soluble and assimilable bodies required for the nutrition of the plant, but also build them up synthetically, as, for example, in the elaboration of starch from sugars.



**Preparation and Properties.**—Enzymes are protein substances, mostly uncrystallisable, chemically coagulated by heat, and precipitated by alcohol. They are all soluble in water; their composition is very similar, and their activity is destroyed by coagulation. This occurs at varying temperatures.

It is difficult to obtain enzymes in a condition of sufficient purity for satisfactory analysis, and as a consequence the exact composition of many of these bodies and their precise properties remain very doubtful. They are, however, similar, in that they can all be precipitated under certain conditions by alcohol and many neutral salts as phosphate of lime, carbonate of magnesia and alumina, and such substances form the basis of their preparation and separation from each other by a process known as "salting out."

It is not known how such precipitation acts, but no doubt it is largely mechanical. It is, however, a curious fact that whilst agitation alone will to a large extent separate out these bodies, yet under certain conditions excessive agitation will actually kill them.

Most enzymes contain nitrogen, though the percentage appears to vary considerably, probably due to the above-mentioned difficulty of obtaining them in a pure condition. A certain class of enzymes, however, known as the oxidases, appear to contain no nitrogen, whilst it has also been suggested that at any rate some enzymes contain both nitrogen and carbohydrate in their molecules, and if this is so they belong to a group of bodies hitherto unknown, or certainly extremely rare, in organic chemistry. The



percentage of ash also varies quite appreciably in the different enzymes analysed, and phosphate of lime, its most important constituent, is usually present in considerable quantity.

**Test for Enzymes.**—Enzymes will decompose hydrogen peroxide, which, therefore, serves as a useful test for them. To carry out the test, proceed as follows :—

Prepare a 1-per-cent. solution of gum guaiacum in absolute alcohol, and to a few cubic centimetres add two or three drops of hydrogen peroxide solution. If any cloudiness results, add absolute alcohol until the liquid clears. The red guaiacum solution, so prepared, if added drop by drop to any liquid containing enzymes, will be transformed into an intense blue coloration due to the formation of a dye. As the action is an oxidising one, no other oxidiser must be present in the liquid, and in the absence of such the change of colour will confirm the presence of enzymes.

**Classification.**—Enzymes may be roughly classified as follows :—

*Group 1.—Hydrolysing Enzymes :—*

Diastase producing maltose and dextrin from starch.

Invertase       ,,       invert sugar from cane sugar.

Maltase         ,,       dextrose from dextrin and maltose.

Lactase         ,,       dextrose and galactose from lactose.

Cytase          ,,       various sugars from cellulose.

*Group 2.—Proteolytic Enzymes,* or pepsin bodies, as peptase, which convert insoluble nitrogenous substances into soluble forms already discussed in Part I, namely, the proteoses, peptones, and amides, whilst the complete

breaking down of insoluble nitrogen is effected by, at any rate, two specific enzymes, viz. :—

Trypsin, transforming protein into proteose.

Pepsin, transforming proteose into peptones and amides.

*Group 3.—Enzymes of Oxidation.* It is unnecessary to consider the members of this group, but many of them take part in colour reactions.

*Group 4.—Decomposing Enzymes.* Zymase, converting sugar into alcohol and carbonic acid.

## DIASTASE OR AMYLASE.

Diastase is a soluble nitrogenous body, first discovered by Kirchoff in gluten. It hydrolyses starch to maltose and dextrin, and is found in nearly all cereals, principally in barley, but also to quite a considerable extent in oats, rice, and maize. It has also been found in some moulds, but in nothing like so large a quantity as the enzyme invertase presently to be described. Diastase is present both in malted and in unmalted grain, but there appears to be considerable difference in its character when derived from these two sources. Thus the temperature of optimum activity is different in the two cases, and the power of barley diastase in liquefying gelatinised starch is much less than malt diastase, whilst it is quite unable to corrode starch granules, a power which is possessed to some extent by malt diastase.\*

\* For some further work upon barley diastase, see a paper by J. L. Baker, *Journ. Chem. Soc. Trans.*, 1902, p. 1177.

It is highly probable that malt diastase does not consist of one body, but a mixture of several, and it has at least two perfectly distinct functions: it liquefies starch and also converts starch into maltose and dextrin. At present these two supposed separate enzymes of diastase have not been isolated from one another, no doubt because of the very great experimental difficulty involved, but there is little doubt that the liquefying power of diastase is due to a different enzyme from that which saccharifies starch, for the former action continues at temperatures considerably above those at which any saccharifying action occurs. The saccharifying action of diastase is exerted at any temperature above 40° F. Its maximum energy is attained at a little below 130°, and immediately the temperature is raised above this, its saccharifying power is diminished. At 150° considerable restriction occurs, whilst at 167° its action is almost entirely arrested, and at 176° it is absolutely destroyed. This diminution in power is accompanied, and indeed produced, by the coagulation of its albuminoid constituents, for, commencing at 135° F., a steadily increasing proportion is coagulated until, at boiling point, the whole is thrown out of solution. The liquefying power of diastase is, on the other hand, retained up to temperatures of 200°, slightly, indeed, at a temperature of 205°. Thus it happens that if malt is added to gelatinised raw grain at a temperature of say 200°, the liquid will be thinned down, owing to the transformation of gelatinised into soluble starch, but no saccharification will take place. The practice of using a small proportion of malt in a hot raw-grain converter is based upon this fact.

Not only do these properties of diastase suggest the action of two enzymes, one saccharifying and one liquefying, but it is further supposed by Duclaux and others that the saccharifying diastase is itself made up of two enzymes, one producing maltose from starch, and the other dextrin. The theory of a double enzyme in the saccharifying diastase is further emphasized by the fact that when diastase solution is heated to temperatures varying from  $140^{\circ}$ — $150^{\circ}$  and afterwards cooled back, its issue is different to diastase not so heated, for if the two extracts are allowed to act upon starch under the same conditions it will be found that the originally heated diastase retains its activity with regard to the percentage of maltose produced *only up to a maximum of 30 per cent.*, and though it will still convert starch up to this amount, it is quite unable to continue its further hydration. On the other hand, the unheated diastase will produce much larger percentages of maltose and will carry the conversion to a further degree. Such experimental facts strongly suggest that the saccharifying diastase is actually made up of at least two different enzymes.

Assuming that the saccharification of starch is thus due to two specific enzymes, the suggestion has been made that the enzyme producing dextrin from starch should be known as "amylase," and the enzyme producing maltose from dextrin, as "dextrinase."

The variations in the final product obtained when diastase solution is heated as described above might be explained by assuming that each of these enzymes is differently affected by temperature, that is to say that

amylase is less retarded by a temperature of about 155° F. than is dextrinase, so that at such temperature dextrin is more easily formed than maltose, whilst with lower temperatures the reverse is the case. Whilst, however, there is a great mass of experimental evidence bearing upon the peculiar action of diastase, or the group of ferments of which it is made up, no theory has yet been advanced which is entirely satisfactory.

**Accelerating and Retarding Substances.**—The saccharifying power of diastase is affected by the presence of other substances in solution, and, like all other enzymes, it is very sensitive to alkalinity, a slightly acid or alkaline solution producing great differences in diastatic activity, and whilst a slightly acidulated liquid will distinctly accelerate its action, an alkali such as sodium carbonate will strongly retard it. According to Effront 5 milligrammes of sodium carbonate in 100 c.c. of neutral starch solution will diminish the diastatic activity by 20 per cent.

Certain substances of a preservative character, such as alcohol, salicylic acid and formaldehyde, will diminish diastatic activity.

Calcium chloride solution in excess of 1 per cent. also retards, decreasing the diastatic activity by 50 per cent. On the other hand the same author has shown that the presence of asparagine, ammonium acetate, phosphoric acid, and ammonium phosphate increases its action; with amides this is particularly marked. Asparagine, the best known substance belonging to that class, is found to assist diastatic activity in such a way as to considerably increase the

percentage of maltose formed. Thus according to Effront,\* as comparative results :—

0·005 gramme of asparagine will result in 25·5 per cent. maltose.

0·02 gramme of asparagine will result in 37 per cent. maltose.

0·05 gramme of asparagine will result in 61·2 per cent. maltose.

1 gramme of asparagine will result in 61·2 per cent. maltose.

But amides increase diastatic activity only when the diastase is working under restricted conditions. With maximum diastatic activity the addition of amides does not appear to appreciably increase the percentage of maltose formed. Phosphates, such as ammonium phosphate and acid calcium phosphate, act in a similar way.

Ford and Guthrie† regard the maximum diastatic activity as taking place in strictly neutral solution, and suggest that Effront's results are based upon an imperfectly neutral starch or enzyme solution. They consider that neither amides nor phosphates exert any accelerating action upon diastase, except that due to any slight acidity which such substances may show.

The presence of a cold infusion of raw grain also accelerates action, and this occurs even when the infusion has been previously boiled. The speed with which starch is saccharified increases with the temperature

\* *Enzymes and their Applications*, Effront (Prescott), 1902.

† *Journ. Chem. Soc. Trans.*, 1906, vol. 89, p. 76.



of saccharification, as shown by Erich in the following table :—

| Temperatures of<br>Saccharification. | Time employed for<br>Saccharification. |
|--------------------------------------|--|
| 140° F.                              | 120 minutes.                           |
| 149° F.                              | 25–30 „                                |
| 158° F.                              | 10 „                                   |
| 167° F.                              | 10 „                                   |

If, therefore, it is desired to rapidly saccharify starch, temperatures should be adopted above that at which the maximum degradation of starch takes place. But although, as shown by the preceding table, we obtain a ten times more rapid conversion at 158° than at 140°, yet the transformation products will be quite different in the two cases, for, speaking generally, the lower the temperature of saccharification, the larger the amount of fermentable sugars produced.

**Preparation.**—It is very difficult to satisfactorily separate diastase. C. J. Lintner has prepared it from malt in the following manner :—

Air-dried malt is ground, and digested for about 24 hours, with two or four parts by weight of 20-per-cent. alcohol. The liquid is filtered, and two or three times its volume of absolute alcohol is added. This produces a white precipitate, possibly consisting of, but certainly containing, diastase. The precipitate is collected, washed—twice with absolute alcohol, then with ether—and finally dried in a vacuum. The substance thus obtained is a white powder, which readily dissolves in water, forming a clear solution.



Payen has observed that diastase thus prepared contains some mineral matter. If steps are taken to re-purify the diastase, it is found that as the mineral matter is reduced, so the activity of the diastase is lessened, and that therefore the purer the diastase the less its activity.

This result, however, is more likely to be due to the retarding action of the alcohol used in the preparation or some other cause, and Wroblewsky more recently prepared diastase by salting out with ammonium sulphate, instead of precipitating with alcohol.

The ammonium sulphate was added as a 50-per-cent. solution until a yellow precipitate was separated, and this was afterwards filtered off and washed with 54-per-cent. ammonium sulphate solution. The diastase thus prepared was extremely pure and very energetic, and the nitrogen percentage was 16·5, an amount considerably higher than that obtained from Lintner's preparation, which gave only 10·4 per cent. of nitrogen.

## INVERTASE.

This enzyme, sometimes called sucrase, hydrolyses cane sugar into invert sugar according to the equation :—



It is found in malt, and in both the animal and vegetable kingdoms, in most types of yeast, and also in many moulds. C. O'Sullivan and Tompson\* have carefully

\* *Journ. Chem. Soc.*, 1890, pp. 834—931.

examined its properties. It is most active at 131° to 140° F. At 150° it is gradually, and at 167° it is instantly, destroyed. The presence of acid, particularly sulphuric acid, accelerates its action, but if the acidity exceeds a certain limit, it very powerfully restricts the enzyme. Caustic alkalies immediately destroy its action. Its power of hydrolysis is very great.

The enzyme may be prepared in several ways, and it is only necessary to allow pure pressed brewers' yeast to stand for a month or so until it has become quite liquid with no further fermentative power, when it will be found to be strongly invertive. The enzyme itself can be separated from this liquid by precipitation with alcohol, though O'Sullivan and Thompson, who first attempted this method, did not find it possible to carry the purification very far, as all their efforts in this direction resulted in destruction of the invertase.

The difficulty of separating the enzyme in a pure state is largely due to the fact that in many cases it is strongly retained in combination within the cell walls, and when so found it is very difficultly soluble in water. According to Fernbach, different types of yeast show very considerable variations in their invertase activity, some types being even as much as fifty times more active than others. As in the case of most enzymes its formation is favoured by soluble nitrogenous compounds such as peptone, but it is curious that phosphates, which are powerful adjuncts to yeast in its fermentative action, are not favourable to invertase activity.

## MALTASE.

This enzyme is present in yeast, and possesses the power of transforming the maltose of malt wort into dextrose, as a preliminary to fermentation. The substance is also probably present in small quantities in malt, and has been found in most cereals, particularly in maize, though the enzyme as secreted by yeast appears to differ in its properties from the enzyme in maize, a difference explained by the probability of interfering foreign substances in the diastase as prepared from each of these two different substances. Yeast maltase is practically destroyed at a temperature of 100° F. It is known that an increase in nitrogenous food will considerably strengthen the supply of maltase produced, but at present very little is known as to the action of this enzyme.

## LACTASE.

Lactase is found in a few yeast-like organisms which are more probably *Torulæ*. It is the enzyme producing lactic acid from milk sugar or lactose, a substance which is particularly liable to lactic fermentation. But this can only take place from lactose in the presence of the enzyme.

## CYTASE.

This substance has been discovered and examined by Brown and Morris, who found an enzyme to be present in germinating barley, possessing the power of corroding the cellulose walls of the starch granule, and to this substance

the name of cytase has since been given. Its action in malting is of the utmost importance, for its office is to prepare the starch granule for subsequent transformation under the influence of diastase, and the "tenderness" of a malt is to a large extent due to this cause. A. R. Ling\* has shown, however, that much of the cell wall remains intact after modification.

Cytase is present in green malt, but is destroyed during ordinary kiln drying. Its maximum activity is stated to be at 104° to 111°. It is much crippled at 122°, and is completely destroyed at 140°. The writer believes that cytase occurs in kiln-dried malts, provided the temperature be sufficiently low, for with cold infusions of samples of malt, prepared for a special purpose, the temperature of which did not exceed 130° on the kiln, he was able to prove very marked erosive action on the cell-walls of starch granules. It seems somewhat doubtful whether the product of its action is soluble starch. No definite information upon this point appears to have as yet been published.

## PROTEOLYTIC ENZYMES.

Very little is known of these bodies. There is certainly a proteolytic enzyme in germinating barley. This was suspected for some time, but has only definitely been proved in recent years. It is present also in kilned malt, and probably consists of two enzymes, comparable to peptase and tryptase as obtained from animal organs.

These enzymes possess the power of hydrolysing certain proteins, and the particular character of the degradation or

\* *Brew. Journ.*, 1904, vol. 40, p. 741.

resulting soluble nitrogen compounds appears to depend upon the temperature. The maximum proteolytic action in the mash-tun is probably at 120° to 125° F., and not much action takes place at temperatures above 145°. The superior strength of yeast grown in a wort which has been produced at low mashing temperatures is, as has been previously explained, probably due in this way to the peptonisation of certain proteins during the mash-tun treatment, and such distinctive substances as the proteoses and peptones are certainly obtained by the degradation of protein bodies, whilst there is no question that the action of these enzymes has a very important influence in rendering the wort suitable for the growth of the yeast.

It has been suggested that proteolysis is of far greater importance in the germinating of barley than was originally supposed, and it is the opinion of some that it takes a larger share in obtaining completeness of modification than does cytase in its action of breaking down the cell walls of the starch grains.

A method has been suggested by P. Schidrowitz\* for ascertaining the proteolytic activity of malts, which depends on the liquefactive property of peptase when acting upon gelatine.

The practical value of such a test would undoubtedly be very considerable, and the method suggested by the author should prove a satisfactory basis for proteolytic determination in malt, but as Schidrowitz himself pointed

\* "Some Experiments on the Proteolytic Enzyme of Malt," *Journ. Fed. Inst. Brew.*, 1903, p. 361; and also *Journ. Fed. Inst. Brew.*, 1904, p. 166.

out, the results of his determinations do not for the moment allow of such interpretation as would prove of practical value in malt analysis. Some proteolytic figures are given with comparative malt analysis in the *Journ. Inst. Brew.*, 1909, p. 592.

## ZYMASE.

Until 1879 the phenomenon of fermentation was always considered to be dependent upon the living organism yeast, and though it was believed that some form of enzyme or enzymes was responsible for fermentation, it was thought that these could not react with sugar, producing alcohol, carbonic acid, and other products, apart from the living cell.

By submitting yeast cells to great pressure Buchner in 1897 revolutionised these ideas, by obtaining a liquid expressed from the yeast, which gave rise to alcoholic fermentation without the presence of any living yeast cells. This discovery was not only very important from the point of view of alcohol fermentation alone, but it immediately suggested that phenomena of a like nature, such as the various fermentations produced by bacteria, might also be brought about by enzymes excreted by these organisms in much the same way that enzymes are secreted by the yeast cells. Fermentations of this kind caused by bacteria are the butyric, acetic, etc.

As might have been supposed, this discovery of Buchner at once suggested to the industrial world that it might be possible to carry out fermentation in the brewing, distilling, and other industries of a like nature by using zymase

itself, so getting rid of the many difficulties obtained where living organisms like yeast were employed.

Whether such a revolution in industrial fermentation will ever be brought about is only a matter of conjecture, but at the present moment the practical difficulties which would arise appear to be insuperable. Thus, for example, the yeast cell possesses many valuable properties other than that of merely fermenting sugar into alcohol and carbonic acid gas. The maltose, by far the largest sugar constituent of wort, would have, of course, to be treated with the enzyme necessary to render it fermentable, and, further, the dextrins and malto-dextrins present in wort and beer would require to be similarly treated. There is, too, the obvious drawback that it is not a question of adding a pure preparation of zymase, but a highly nitrogenous and quickly putrefactive expressed yeast juice. Such juice is in some cases a hundred times less than the yeast itself in fermentative power, so that the volume of liquid juice required for a given fermentation would be considerable. It will, too, very rapidly fall off in fermentative energy, and, indeed, begins to weaken a few hours after its preparation.

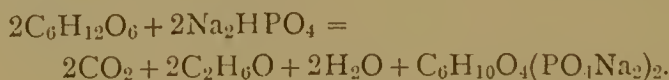
Apart from the added fact that the all-essential withdrawal of certain nitrogen compounds from the wort by the growing yeast would be absent in the case of the yeast juice, the question of infection from other organisms, such as wild yeast or bacteria, would appear to be a very serious one. These and other drawbacks make the possibility of the practical use of zymase in the brewery a remote one.

Buchner and Meisenheimer stated, in 1904, that



alcoholic fermentation of sugar is brought about by two distinct enzymes; that, in the first place, the enzyme known as zymase converts sugar into lactic acid, and that afterwards a second enzyme lactacidase acts upon the lactic acid and breaks this down into alcohol and carbon dioxide. This is an interesting point as further illustrating the fact so often met with that several enzymes appear to act together in a system, but this theory has recently been disproved, and consequently relinquished, by Buchner himself.

A. Harden,\* in some recent experiments with yeast-juice, has found that the addition of almost any soluble phosphate to yeast juice in the fermentation of sugar will very considerably increase the rate of fermentation, and whereas yeast juice possesses only about 1/40th the fermentative power of living yeast cells, phosphates will increase this up to one-half. Further, he made the important discovery that up to a certain point there was a definite chemical relation between the phosphate added and the amount of sugar decomposed, making it evident that a true combination of sugar and phosphate was obtained, which substance he named hexose-phosphate, with formula  $C_6H_{10}O_4(PO_4H_2)_2$ , and suggested the following equation as representing the change which takes place:—



The hexose-phosphate is not a true intermediate product, but it does, nevertheless, continually split up into a sugar

\* "Recent Researches on Alcoholic Fermentation," by Arthur Harden, *Journ. Inst. Brew.*, p. 623, 1910.

and phosphate, re-forming again when fresh sugar is added. Hexose-phosphate appears to be produced by an enzyme known as hexose-phosphatase.

Harden had previously found that boiled and filtered yeast juice, when added to yeast juice, stimulated the fermentative energy, and he attributes this to the work of a co-enzyme, the composition of which is not yet known, but which appears to be closely related to, though his experiments prove that it is definitely not, a phosphate.

From expressed yeast juice two substances may be separated by rapid dialysis, both of which are essential for producing fermentation. Neither of these substances so obtained can separately ferment sugar—combination is essential. The substance which passes through the dialyser is known as the co-enzyme, and even when this co-enzyme is boiled, it will, in combination with the residue containing the true enzyme, and which does not pass through the dialyser, ferment sugar.

The fact that fermentation of sugar by means of yeast juice soon becomes exhausted has been explained by Buchner as due to the presence of a proteoclastic enzyme present in the juice which destroys the zymase, or other enzymes of fermentation. Dr. Harden thinks the destruction is more likely to be due to one of the fat-decomposing enzymes, such as, for example, lipase, rather than to one of the nitrogen-degrading or proteoclastic enzymes. He concludes this most important paper by the following summary :—

“ If we sum up the position of affairs so far, it is that ever since the discovery by Buchner of yeast juice, matters have

been getting constantly more and more complicated. It has been found that the yeast cell does not simply contain an alcoholic enzyme which can be compared with a substance like invertase, but that it is also provided with a regular army of enzymes and co-enzymes ; that fermentation involves the presence of phosphates ; that with each of these enzymes there is provided another enzyme which gradually destroys it. Moreover, Buchner has recently established the fact that the cell also contains a substance which protects the enzyme from the proteoclastic enzyme."

The actual facts which underlie the action of the zymase ferment are thus at present very imperfectly known, and, as in the case of diastase in its action upon starch, will require an enormous amount of experimental work before the problem of its composition and mode of action is solved, and meantime many theories will no doubt be formulated in explanation.

It will have been seen from the above short sketch of enzymes and their action, in so far as they are of importance to the student of brewing, that they represent, perhaps, the most difficult problem that the chemist has yet attempted to investigate.\*

\* See also "Alcoholic Fermentation," by Arthur Harden, *Mono-graphs on Bio-chemistry*, 1911.

### Part III.—CARBOHYDRATES.

These may conveniently be divided into three groups, as follows :—

- |                           |   |
|---------------------------|---|
| I. Cellulose              | } with formula $n C_6H_{10}O_5$ .       |
| Starch                    |   |
| Dextrin                   |   |
| II. Maltose               | } with formula $n C_{12}H_{22}O_{11}$ . |
| Cane Sugar (or Sucrose)   |   |
| Lactose                   |   |
| III. Dextrose (Glucose or | } with formula $n C_6H_{12}O_6$ .       |
| Grape Sugar)              |   |
| Levulose (or Fructose)    |   |
| Invert Sugar              |   |

*Group I.*—The carbohydrates of this group are of high molecular weight, and are split up by hydrolysis ; that is, by the addition of molecules of water into the simpler members of Groups II and III. This hydrolysis may be brought about by enzymes or acids. The group are known as the Polysaccharides or Polyoses.

*Group II* are known as the Disaccharides or Bioses, and contain 12 carbon atoms. They are also hydrolysed by enzymes and acids.

*Group III* are the Monosaccharides or Monoses, and they may have 6 or 5 carbon atoms, being then known as Hexoses and Pentoses respectively. They may be considered as carbohydrates revertible to the members of Group I by dehydration or loss of water, as  $nC_6H_{12}O_6 - H_2O$ .

The above-mentioned carbohydrates comprise only a few members of each group, but are sufficient for our purpose.

## GROUP I.

## CELLULOSE.

This is the main constituent of the cells of which all vegetable structures are composed. It forms a great portion of the husk of barley and other cereals, and of the external tissue of yeast, but in this case it differs from the true cellulose of cereals. It is colourless and transparent, perfectly insoluble in water or alcohol, but is dissolved by an ammoniacal solution of  $\text{CuO}$ , from which it is precipitated on the addition of acids in the form of a white flocculent precipitate. The purest form of cellulose is obtained by extracting Swedish filter paper with hydrofluoric acid to remove traces of silica, afterwards well washing and drying at  $100^{\circ}$ . Cellulose is coloured yellow with iodine. When treated with cold concentrated sulphuric acid, it is at first converted into a colourless jelly-like substance, which gradually dissolves in water, presenting the characteristics of dextrin. If much water is added, and the solution boiled for four or five hours, the cellulose becomes converted into glucose. Cross and Bevan have shown that a modification of ordinary cellulose exists in many vegetable tissues, including those of barley and malt. These are named the oxy-celluloses, which are convertible by means of sulphuric acid into a fermentable sugar, which, however, probably is not dextrose. It has been proposed to take advantage of this fact, and to convert the cellulose in brewers' grains, left after the removal of the wort, by boiling them with acid in the mash tun, then neutralising with chalk, and washing out the fermentable extract thus obtained and adding it

to the fermenting tun. Of course, this process could not be adopted in iron or copper mash tuns, nor in those provided with metal rakes.

## STARCH.

Starch when examined under the microscope is found to possess an organised structure, consisting of an outer coating, termed "starch cellulose," and an internal portion, termed "granulose." Brown and Heron have stated that starch cellulose consists of two substances, one easily converted into soluble starch on boiling, the other unchanged by ordinary boiling, but also converted into soluble starch when boiled with an alkali.

Starch granulose is, so to speak, imprisoned within the cell wall of the starch, and until this is ruptured it cannot either enter into solution or be acted upon by diastase. Granulose may be liberated from starch by attrition, by raising a mixture of it and water to such a temperature that the cells burst, or in other ways. The granulose thus obtained gives the characteristic blue colour of starch with iodine. It is doubtful whether on such liberation of the granulose it enters into true solution, and there is considerable difference of opinion on this point. Brown and Heron have expressed the opinion that the granulose is actually in a true state of solution, and that the viscosity of starch paste is due to the swollen state of the cellulose. This viscosity varies in a strange manner. Much depends upon the temperature at which the starch is dried, and the duration of the drying operation. The longer starch is dried, and the lower the heat at which this has been

effected, the more viscous will be the solution, and the difference in viscosity due to different methods of preparation is stated to amount to more than 3 to 1. Moritz and Morris are of opinion that, on boiling starch, the granulose is converted into soluble starch. The gelatinising point of starch varies greatly. The following temperatures are those of Lintner,\* which are much higher and probably more correct than those of Lippman :—

|                |     |     |     |         |
|----------------|-----|-----|-----|---------|
| Potato Starch  | ... | ... | ... | 149° F. |
| Barley Starch  | ... | ... | ... | 176° F. |
| Oat Starch ... | ... | ... | ... | 185° F. |
| Rice Starch    | ... | ... | ... | 176° F. |
| Maize Starch   | ... | ... | ... | 167° F. |
| Wheat Starch   | ... | ... | ... | 176° F. |
| Rye Starch ... | ... | ... | ... | 176° F. |

Starch obtained from different sources varies in its size and form, and an examination under the microscope will frequently enable the observer to ascertain the source from which it is derived. Starch is commercially prepared from grain, by steeping it in water for a considerable time, when the lactic acid—always developed under such conditions from the sugar which is present—disintegrates and partially dissolves the nitrogenous matters, facilitating also the separation of the remaining insoluble nitrogenous matters. The matter thus obtained is repeatedly lixiviated with water, and the starch by reason of its high specific gravity is separated by subsidence. Latterly another and more successful process has been employed, in which a dilute

\* *Brauer. und Malzer. Kalendar*, 1880.



solution of caustic soda (half an ounce per gallon of liquid) is used to dissolve the gluten.

Ungelatinised starch is not acted upon by diastase,\* but after gelatinisation is attacked by this substance readily at all temperatures below the point at which diastase is itself destroyed. When starch is heated in an acid solution, it becomes hydrated into a mixture of dextrin, maltose, and dextrose, and if the process is carried sufficiently far, dextrose alone is formed; 90 parts of starch become 100 parts of dextrose after inversion. If, however, the heating of acid is continued beyond this point, further hydration occurs, and a substance known as gallisin is formed.

When starch is heated to a temperature of about 500° F., it acquires a yellowish tinge, becomes soluble in water, and is converted into a mixture of soluble starch and dextrin. This preparation is much used as British gum, which may be also prepared by moistening starch with dilute nitric acid, allowing it to dry, then spreading it out in thin layers and raising it to a temperature of 250° F. Some of the malt substitutes offered to brewers consist practically of this substance.

The products of the transformation of starch, or more properly soluble starch, by diastase have been the subject of a large amount of research, and, as was necessarily the case when dealing with such a complex problem or series of problems, many of the original theories advanced have had to be modified as the result of later work. It is not possible to give here any detailed statement of the results arrived at

\* Under certain conditions this does not appear to be true.

by different observers, but some of the main facts may be summarised as follows :—

**Starch Hydrolysis.**—The products resulting from the action of diastase upon starch will vary with—

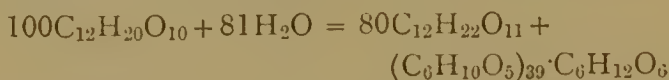
- (1) The temperature at which conversion takes place ;
- (2) The time taken for completing the reaction ; and
- (3) The particular starch employed.

With unrestricted diastatic action and under the most favourable conditions of temperature for enzyme activity, Brown and Morris\* showed that a definite resting stage in the hydrolysis of starch was undoubtedly obtained when maltose and dextrin were produced with percentages of 84·4 and 15·6 respectively. The equation representing this stage was given as—



Further investigations,† however, disclosed the fact that the formula for the so-called stable dextrin was not correctly given by the equation, and that the molecule of this substance was at least twenty times as great, the formula being  $40\text{C}_6\text{H}_{10}\text{O}_5 + \text{H}_2\text{O}$ .

This necessitated a change in the equation, increasing at the same time the molecular value of starch, thus—



which probably more correctly expresses the change that takes place, and includes one member of the  $\text{C}_6\text{H}_{12}\text{O}_6$  group.

\* *Journ. Chem. Soc.*, 1889.

† Brown and Millar, *Journ. Chem. Soc. Trans.*, 1899, p. 333.

This resting stage in hydrolysis is a well-defined and important one, but the stable dextrin could only be purified with considerable difficulty, and, even when purified as far as possible, it was still found to have a small reducing power, proved by the authors as not, at any rate, due to free maltose, since no osazone was obtained by treatment with phenylhydrazine, nor was it in their opinion due to a small percentage of a malto-dextrin compound.\*

Under these conditions of diastatic conversion, the optical activity and reducing properties of the products obtained by the equation correspond to  $[\alpha]_D$  149.2 and  $R^\dagger$  81.8, figures which very closely approximate to those obtained by actual experiment. The empirical formula of starch would thus be  $80C_{12}H_{20}O_{10} \cdot 40C_6H_{10}O_5$ , exemplifying the fact that on hydrolysis four-fifths of the starch molecule yields maltose and one-fifth a stable dextrin.

With restricted diastatic action, however, much more complex results are obtained, and where the diastase is weak in proportion to the amount of work it is required to do, the starch of more stubborn character, or the temperature high, the fermentable maltose declines from its maximum and instead a series of substances known as the malto-dextrins are obtained. These substances have given rise to the malto-dextrin theory, and this series of bodies are conveniently regarded as chemical compounds. However this may be, malto-dextrins differ from both free

\* Quite recently (*Journ. Inst. Brew.*, 1909, p. 632) A. R. Ling expresses the opinion from some unpublished work that the maltose obtainable by the above method is less than that shown by the equation and that malto-dextrins are also present.

† For explanation of R and K, see p. 103.

maltose and dextrin in that they form a series differing in the extent to which they are hydrolysable by diastase, and consequently in the extent to which they are fermentable.

With the maximum restriction maltose is formed in the smallest quantity, and malto-dextrins of the highest type, these substances being very difficultly hydrolysable, nearly unfermentable, and approaching the stable dextrin in their general properties, a type exemplified by the symbol M1/D19.

As the restriction becomes less a greater portion of so-called lower type malto-dextrins are formed, down to the very weak combination of 19 maltose to 1 dextrin, exemplified by the type M19/D1, and finally with unrestricted diastase stable dextrin and free maltose are formed as described by the equation given above.

Without discussing what these malto-dextrins really are, it is certain that substances of this nature are formed under the conditions mentioned, and that they are ultimately hydrolysed by further diastatic action to free maltose, so that a wort produced under the average conditions of brewing, that is, with restricted diastatic action, will contain free maltose, stable dextrin, and a series of malto-dextrins varying in amount and fermentability.

It should be noted that these malto-dextrins will not ferment simply by the action of the enzymes present in yeast, but require also diastatic enzymes to effect their decomposition, such as, for example, malt flour. Dextrin on the contrary cannot be hydrolysed even under these conditions, but appears to require the addition of both diastase and yeast ferments working together, and under these

conditions it is ultimately hydrolysed and rendered slowly fermentable.

The acceptance of the above explanation involves the acceptance of the malto-dextrin theory also, a theory which has been strenuously questioned by many, but which has certainly not yet been disproved. On the contrary, the accumulation of evidence appears to further substantiate the theory of Brown and Morris ; and though undoubtedly the last word has not yet been written on the subject, we may safely accept the theory as a working hypothesis, whilst quite recognising (as, of course, do the authors themselves) that we have not yet reached finality in that, as in so many other of the problems connected with brewing.

The malto-dextrins possess the following distinguishing characteristics :—\*

1. They give numbers, on analysis, which allow their composition to be expressed in terms of a mixture of maltose and dextrin.
2. They cannot be separated by any known chemical methods into maltose and dextrin.
3. They are completely converted into maltose by the action of malt extract or diastase.
4. They are unfermentable during the primary fermentation.

The same authors are of opinion that during primary fermentation the free maltose is entirely fermented away, that during storage the malto-dextrins are gradually

\* *Trans. Lab. Club*, 1890, p. 83.

hydrolysed and fermented, but that the free dextrin remains practically unaltered even on prolonged storage.

Starch is a difficult substance to free entirely from moisture, and this is particularly so with the residual portions, to such an extent as to indicate that a percentage of moisture is chemically combined in the starch molecule.

Again, the starch cell is built up by concentric layers working from without inwards (and each layer so formed appears to differ from its fellow in the amount of water it contains), and it is of interest to note that according to some authorities, particularly Duclaux, the difference in the properties of the dextrin formed is attributable directly to the fact that the complex molecules of starch are made up of layers which are uneven in their structure and homogeneity, and offer therefore, even when the starch is liquefied, different resistances to the enzyme's activity. The theory is somewhat strengthened by the well-known fact that starch from different cereals will give different transformation products when acted upon by diastase, thus, for example, potato starch is much more readily saccharified than is barley starch. On this view the dextrins are not so much distinct chemical substances, but rather differ in their properties owing to the fact that they are formed under varying conditions, due to the complex condition of the starch.

A summary of the "Chemistry of the Dextrins during the last Twenty-Five Years" has been given by O. Mohr (*Wochenschrift Brauerie*, 1908), and an abstract may be found in the *Journ. Inst. Brew.*, 1909, pp. 111-122. Reference to the great mass of work on the question of starch conversion may be found in *The Principles and Practice of Brewing* (W. J. Sykes and A. R. Ling, 1907).

**Soluble Starch.**—This body is that first formed in the degradation of starch by heat, acids, or diastase. It may be conveniently prepared by the method of Lintner, and is commonly used in the determination of diastase by Lintner's method. The method consists in treatment of starch with a 7·5 per cent. solution of hydrochloric acid for seven days, but if stronger acid (12 per cent.) is used, the change is complete in 24 hours. The starch granules undergo no apparent physical change, retaining their original appearance when examined under the microscope.

Its opticity is about  $+202[\alpha]_{D3-86}$ ; it does not reduce Fehling's solution, and forms, with iodine, an intense deep blue coloration, due to formation of iodide of starch. It is nearly insoluble in cold water, but easily dissolves in warm water, precipitating on cooling in a white flocculent condition.

## DEXTRIN.

The production of this body from starch by the action of diastase, dilute acid and heat has already been referred to. It owes its name to its dextro-rotatory power on light; it is a non-crystallisable solid substance, very soluble in water, but insoluble in alcohol or ether, and may be precipitated from its aqueous solutions by the addition of the former.

There has been much confusion as to the existence of various types of dextrin, Greissmayer, O'Sullivan, Nägeli, and others describing several. There is probably, however, only one true dextrin, which possesses a specific rotatory power of  $202[\alpha]_D$ , gives no colour with iodine, and has only a slight reducing power (R 5·7).



It is unfermentable, and is produced, as before described, in the hydrolysis of starch by means of diastase, but is apparently only further hydrolysed very slowly by the combined action of diastase and yeast.

It has long been considered to communicate fulness to beer, but it is probable that its action in this direction has been much over-estimated, and the fulness of a finished beer is probably due more to the presence of malto-dextrin than of actual dextrin. Any fulness which it does communicate is, of course, of a "dry" character.

## GROUP II.

### MALTOSE.

This substance was first noticed by Dubrunfant, but was practically re-discovered by O'Sullivan.\* It is the final product of the action of diastase on soluble starch. It was prepared by him by acting upon gelatinised starch with diastase at about 90° F. Chloroform was added to prevent bacterial growth, and the whole allowed to stand for 10 days. The solution was then evaporated to a syrupy consistency, when the maltose slowly crystallised out and was purified by dissolving in alcohol, and recrystallising. Maltose, when separated from aqueous solution, does so in needle form, and contains one equivalent of water of crystallisation, but when separated from alcoholic solution is anhydrous.

The reducing power of maltose is stated by Brown and Morris† to be  $K_{3.86} = 61$ , which is practically the same

\* *Journ. Chem. Soc.*, 1876, p. 479.

† *Journ. Chem. Soc.*, 1879, p. 619.

as that obtained by O'Sullivan, when allowance is made for the fact that the latter observer worked with the solution factor of 3.85 in place of the now commonly adopted factor 3.86.

The optical activity of maltose is, according to Brown, Morris, and Millar,  $[\alpha]_{D3.86} = +138.0^{\circ}$ .\* Maltose is hydrolysed into dextrose when its solution is heated with dilute mineral acids. It is not acted upon by diastase. Maltose is fermentable by yeast, but only when first hydrolysed into dextrose by a special enzyme contained within the yeast.

Maltose and certain other members of the carbohydrate group are able to react with phenylhydrazine and form osazones, compounds of considerable interest and importance, which are also of some value in the identification of the various sugars. Thus maltose, when its hot solution is treated with phenylhydrazine dissolved in acetic acid, forms such a compound, which separates on cooling as a yellow precipitate which, examined under the microscope, will be found to consist of long, flat, needle-shaped crystals.

Dextrose, presently to be described, forms a similar compound under the same conditions, but the yellow crystals are still more needle shaped, and are sometimes aggregated in a fan-like structure. A further distinction is found in the fact that the maltosazone is much more soluble in hot water than is the glucosazone, which will begin to separate out whilst the liquid still remains hot. The test, therefore, may be used as a means of distinguishing between dextrose and maltose.

\* Expressed as  $[\alpha]_{D17.5}$  and with concentration  $5.7 = +137.8$ , Ling, Eynon and Lane.

## CANE SUGAR OR SUCROSE.

This well-known body, though widely diffused throughout the vegetable kingdom, is chiefly obtained from the sugar cane (*Saccharum officinarum*) and white beet (*Beta maritima* or *vulgaris*).

The former plant contains from 12 per cent. to 20 per cent. of sugar, and the latter from 8 per cent. to 12 per cent. That obtained from the sugar cane is far superior to beet sugar, which possesses, unless very highly refined, a characteristic and unpleasant flavour, a large quantity of salts (notably potassic chloride), together with other disadvantages, rendering it unsuitable for the brewer's use, either alone, or when mixed in any but small proportions with cane sugar.

The difference, however, between cane and beet sugars is not in the composition of actual sugar, but in the impurities which are present; thus it is that when a beet sugar is sufficiently refined, it is quite indistinguishable from cane sugar, but when raw its impurities communicate a most objectionable flavour and smell to the sugar, whereas the impurities of an ordinary cane sugar are not altogether objectionable, but, on the contrary, actually impart a lusciousness which is not obtainable from the purified sugar.

Cane sugar occurs in the juice of all ripe fruits, as also in honey, of which it forms the solid portion, but is associated in these cases with invert sugar—a substance we shall consider presently.

When cane sugar is raised to a temperature of about

300° F., it melts to a clear liquid, which, on cooling, solidifies into an amorphous mass. At a temperature of 320° F. decomposition commences, and the sugar is converted without loss of weight into a mixture of dextrose and levulosan.\* Further increase of temperature converts this into caramel, whilst, with intense heat, gaseous and liquid products are yielded, together with a residue of carbon. Cane sugar itself, or a strong solution of it, when mixed with a little concentrated sulphuric acid, is immediately decomposed, and a mass of carbon formed, which swells to a comparatively large bulk.

When, however, a solution of cane sugar containing not more than 30 per cent. is heated with dilute acid, the sugar, instead of parting with, takes up water, and is hydrolysed into a mixture of dextrose and levulose, to which the name of invert sugar has been given.

The same change is brought about when cane sugar is placed in contact with yeast, or yeast water.

Advantage is taken of the fact that yeast possesses hydrolytic powers upon cane sugar, at temperatures above those at which fermentation is possible, and Thompson has devised a process of inversion in which the solution of cane sugar is digested with yeast at temperatures between 100° and 130° F.

Cane sugar forms insoluble compounds (saccharates) with lime, strontium, and barium, and this property is utilised in processes for the preparation of crystallisable sugar (cane sugar) from molasses and sugar from which the bulk of the cane sugar has previously been separated.

\* Levulosan ( $C_6H_{10}O_5$ ) is entirely unfermentable.

It is not directly fermentable by yeast, but under the influence of invertase it is first inverted and then fermented. Sugar so inverted seems to be capable of more rapid fermentation than when ready-formed invert sugar is presented to yeast, and this irrespective of the fact that commercial invert sugars contain a considerable quantity of unfermentable matters.

Cane sugar is freely soluble in water; a cold saturated solution contains over 65 per cent. of sugar, a boiling solution 82 per cent. It is insoluble in ether, slightly soluble in absolute alcohol. It does not reduce Fehling's solution, and possesses an opticity of  $[\alpha]_{D3.86} + 66.5$ . Its specific gravity is 1.6.

Cane sugar forms no osazone when its solution in water is treated with phenylhydrazine, but after prolonged heating a slight precipitate is formed, which on microscopical examination is found to consist of the characteristic plate form of crystals obtained from dextrose, due to slight inversion of the cane sugar.

## LACTOSE.

Lactose is the sugar of milk; it has the formula



and is present in milk in amounts varying from 4 to 6 per cent. Its specific rotatory power is  $[\alpha]_D + 52.7^\circ$  and it is obtained for commercial purposes as a by-product from milk after the separation of the casein, fat, etc. Lactose is converted by the enzyme lactase into a mixture of dextrose and galactose, and is oxidised by nitric and other acids.

The sugar is very little fermentable, but according to Fisher certain yeasts can ferment it. It is at the moment of some interest to brewers in view of its use in the brewing of so-called "milk stout." Its practical non-fermentability allows of the production of a beer which will retain its sweetness for a longer time than it will when brewed with ordinary glucose or invert sugar. It forms the characteristic yellow crystalline osazone with phenylhydrazine, this being soluble in boiling water, and is similar therefore to maltose but distinguishable from dextrose, in which the osazone is insoluble in boiling water. Lactose is a white non-crystalline substance, very much less sweet than most of the other sugars.

### GROUP III.

#### DEXTROSE. (Glucose or Grape Sugar.)

This sugar exists in grapes and honey, and in the stem and seeds of cereals. Dextrose may be prepared from cane sugar, the product of which, after inversion, consists of equal weights of dextrose and levulose. The solid portion of a commercial sample of invert sugar consists of crystallised dextrose, which may be washed with alcohol, dissolved, and recrystallised from methyl alcohol. It is then separated as hydrate ( $C_6H_{12}O_6 + H_2O$ ). On the manufacturing scale, glucose is prepared from starch-containing materials by means of acid. The substance thus obtained consists of a mixture of dextrose, maltose, and dextrin, or if the hydration has proceeded to its ultimate limit, the whole of the maltose and dextrin is hydrated. In this case, however, it is found that a certain proportion of other substances are



formed. Of the character of these very little is known, but amongst them gallisin most probably exists.

According to Schiebler and Mittelmeier, the unfermentable residue of commercial starch consists of gallisin, which may be separated, and forms a white hygroscopic powder, possessing a cupric oxide reducing power of  $K_{3.86} = 45$ , and an optical activity of  $[\alpha]_{D_{3.86}} = 84$ . The formula  $C_{12}H_{24}O_{10}$  has been assigned to this substance. The unfermentable residue, however, of a commercial glucose does not in the author's experience give figures corresponding to the cupric oxide reducing power and optical activity above named, and either this residue does not consist solely of gallisin, or the reducing and optical powers of that substance need revision. Gallisin is stated to be entirely unfermentable, but converted by heating with acid into glucose. The whole subject is, however, in a very unsatisfactory condition.

Dextrose like maltose forms an osazone and its properties have been compared with maltosazone on p. 93.

Dextrose is readily soluble in water, easily soluble in dilute alcohol, but comparatively insoluble in absolute alcohol. It has only about half the sweetness of cane sugar; it is not charred or blackened on the addition of concentrated sulphuric acid, but if heated with a solution of sodic or potassic hydrate, it acquires a brown colour. On heating to  $340^{\circ}$  F., water is given off, and glucosan ( $C_6H_{10}O_5$ ) is stated to be formed. On still further heating, more water is given off, and a caramelised mass is produced. Dilute nitric acid oxidises glucose into saccharic acid ( $C_6H_{10}O_8$ ). It is also decomposed by heating with anhydrous acetic acid. Dextrose is readily fermentable, and yields 48.67 per cent. of alcohol. It possesses an optical activity of  $[\alpha]_{D_{3.86}} = 51.7$ ,\* and its cupric oxide reducing power is  $K_{3.86} = 100$ . Dextrose solutions, in common with those of some other carbohydrates, possess the phenomenon

\* Ling, Eynon and Lane give  $[\alpha]_{D_{17^{\circ}}} + 52.72$  ( $C = 10$ ).



of muta-rotation.\* A freshly-prepared solution shows an opticity nearly twice as great as that given after standing, and the opticity does not become stationary until after 24 hours, though 2 minutes' boiling, or the addition of a very small quantity of ammonia, at once effects this.

### LEVULOSE. (Fructose.)

Levulose exists in association with dextrose in honey, ripe fruits, etc., and is present in invert sugar to the extent of half its weight. It may be separated by mixing invert sugar with an equal weight of finely-powdered slaked lime, which should be added gradually to the solution of sugars, maintaining the liquid at a low temperature by surrounding the vessel with cold water, to which ice, if necessary, has been added. By this means a milky liquid is obtained, which gradually becomes pasty from the formation of somewhat insoluble calcic levulosate, while the dextrose present forms a compound which is freely soluble, and may be separated by filtration and careful washing. The residue is suspended in water and decomposed by passing carbonic acid through the liquid, when the lime is precipitated as carbonate, and a solution of pure levulose obtained, which may be rendered anhydrous by evaporation *in vacuo*, over sulphuric acid. Thus prepared, it will be found to be a colourless, uncrystallisable syrup, distinctly sweeter and more soluble in alcohol than glucose.

It may be obtained by careful recrystallisation in fine silky crystals, which melt at 203° F.

\* Formerly termed bi-rotation, and first observed by Dubrunfant in 1846.

It rotates a polarised ray of light strongly to the left, hence its name, and this polarisation is more powerful than that of the glucose to the right, so that a solution of invert sugar, containing equal quantities of each sugar, possesses a distinct lævo-rotatory power. Levulose has a less reducing action upon Fehling's solution than has dextrose.

It ferments in contact with yeast, and when heated it is converted into levulosan ( $C_6H_{10}O_5$ ), a body isomeric with glucosan and produced in the same way from glucose by the expulsion of water.

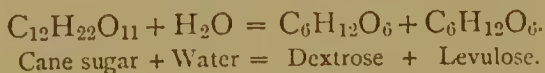
The cupric oxide reducing power of levulose is 92.4, and its lævo-rotatory angle varies considerably with the temperature at which it is determined; at 68° F. this is  $[\alpha]_{D^{38.8}} = -92.0^\circ$ .\*

It is easily decomposed, its solution when boiled rapidly increasing in colour from decomposition.

Levulose forms an osazone with phenylhydrazine, precisely similar to and not to be distinguished in any way from crystals of glucosazone.

## INVERT SUGAR.

This consists of equal proportions of the previously described sugars, dextrose, and levulose. Its formation is a simple hydrolysis of cane sugar, and the following equation expresses the change that takes place—



This hydrolysalation can be readily brought about by

\* Ling, Eynon and Lane  $[\alpha]_{D^{18.5}} = -93.83^\circ$  (C = 10).

enzymes or acids, and the preparation of pure invert sugar is best carried out by Wohl's method\* of treating pure cane sugar with a trace of hydrochloric acid.

Invert sugar is found in nature chiefly in honey and in many fruits.

Commercially it is generally met with as a syrup, and, when solid, its crystallising is due to the dextrose.

Its specific rotatory power varies widely with the temperature and concentration. At about 188° F., invert sugar has apparently no rotatory power at all.

It is readily fermentable with yeast—dextrose being more easily fermentable than levulose.

#### Solution Factor.

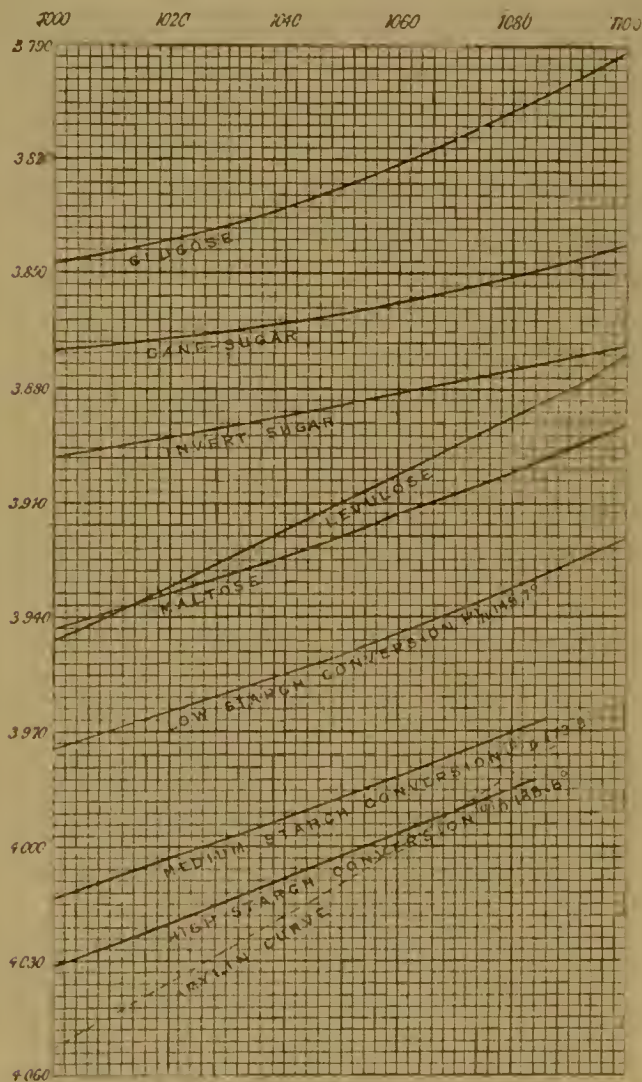
It is frequently necessary in analysis to ascertain the amount of solid matter present in a solution. This might be done by evaporating a known quantity of the solution to dryness, and weighing the residue; but in dealing with the carbohydrates generally, this method is not applicable, because, in the long continued heating which is necessary to remove the last traces of water, decomposition of the organic substance generally occurs. It is therefore customary to ascertain the amount of dry matter present in solutions of the carbohydrates by taking the specific gravity and dividing the excess weight over 1,000 by a factor. The original factor which was for a long time generally used was 3·85. This was based on O'Sullivan's† researches, who found that a solution containing 10 grammes of pure maltose or dextrin in 100 c.c. has a specific gravity of 1038·5. Subsequent work has, however, shown that this factor is not accurate. O'Sullivan afterwards used the divisor 3·95 for starch products, whilst Brown and Heron‡ use the factor 3·86. This factor is correct for solutions of cane sugar having a specific

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\* *Berichte Deutsch. Chem. Ges.*, 1890. See also in its practical aspect a paper by J. L. Baker, "The Preparation of Invert Sugar in the Brewery," *Journ. Inst. Brew.*, 1902, pp. 270-296.

† *Journ. Chem. Soc.*, 1876, p. 129.

‡ *Ibid.*, 1879, p. 605.



(Brown, Morris and Millar, *Journ. Chem. Soc.*, Jan., 1897.)

gravity of 1,050, but is not accurate for starch transformation products. It is, however, generally used, and is adopted in this work. Brown, Morris, and Millar\* have lately given divisors for carbohydrates at various densities, and this table is here reproduced.

For all ordinary purposes, however, it is sufficient to adopt the factor 3.86.

### Cupric Oxide Reducing Power.

This may be stated to be the specific cupric reducing power of a substance referred to dextrose as a standard of 100, and such a figure is indicated by the letter K. Thus  $K = 50$  signifies a substance having half the reducing power of dextrose. Since in technical analysis the amount of reducing sugar is almost invariably determined by means of its solution factor, it is convenient to add the divisor which has been used, thus  $K_{3.86} = 50$  shows that the reducing power is expressed on solid matter determined by the factor 3.86. Brown, Morris, and Millar have proposed to refer the reducing power of sugars to maltose taken as 100, and for indicating this they use the letter R; Thus  $R_{3.86} = 50$  would indicate that the substance under examination had a reducing power of half that possessed by maltose, when the amount of the substance is determined by means of the factor 3.86.

The determination of the copper reducing power is of great value in sugar analysis. The cuprous oxide precipitated by the various sugars, as determined by the method described in Chapter V, and weighed as cupric oxide ( $\text{CuO}$ ), is as follows :—

1 gramme  $\text{CuO} = 0.7435$  maltose.

0.4535 dextrose.

0.4715 invert sugar.

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\* *Journ. Chem. Soc.*, 1897, p. 72.

## CHAPTER III.

**MALT ANALYSIS AND INTERPRETATIONS.**

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**Part I.—WHITE MALT.**

THE following are the determinations to be made :—

Moisture,

Extract,

Colour,

Total matters soluble in cold water, including :

Ready-formed soluble carbohydrates,

Soluble nitrogenous bodies,

Mineral matter,

Diastatic power,

Acidity,

Saccharification, and a

Physical examination.

**MOISTURE.**

Accurately tare a small glass dish or copper capsule, which should be about 2 inches in diameter and about 1 inch deep. Crush rather more than 5 grammes of the malt in an ordinary small hand malt crusher, which must, of course, be absolutely dry, although equally accurate results



are obtained by using a small enamelled grinding mill, but if this is used care should be taken that a little of the malt whose moisture is to be determined is first ground through the mill and thrown away previous to grinding each sample. It has been found experimentally that, with the great bulk of malts, very little difference results, whether the malt is ground coarsely or finely; the mill should, however, be set to grind to a moderately fine powder. Transfer the whole of the crushed or ground malt to the tared dish and accurately weigh out 5 grammes, withdrawing any excess weight by means of a spatula. Dry in a boiling water oven for five hours, cool under the desiccator, and weigh.\*

The malt should, both in the first instance after grinding, and when dried, be weighed as quickly as possible, as it rapidly absorbs moisture from the atmosphere. The loss of weight on 5 grammes of malt multiplied by 20 gives percentage of moisture (if any other weight is taken, it can, of course, be easily calculated to percentage by rule-of-three sum).

*Example :—*

|                            |     |     |         |     | Grammes. |
|----------------------------|-----|-----|---------|-----|----------|
| Vessel                     | and | 5   | grammes | of  | malt     |
| weighed                    | ... | ... | ...     | ... | 32·675   |
| Weight after drying        | ... | ... | ...     | ... | 32·516   |
| Moisture in 5 grammes malt |     |     |         |     | 0·159    |

and  $0·159 \times 20 = 3·18$  per cent of moisture.

\* See Report of Malt Analysis Committee, *Journ. Inst. Brew.*, 1906, p. 6.



Some chemists prefer to dry in the water bath for four hours, weigh and dry again for an hour, reweigh and continue this process until the weight is constant. Practically, however, there is very little advantage in this, as it is extremely difficult to get a malt to remain at a constant weight, and it is more satisfactory to take a definite time and regard the weight as final.

Drying the malt in this way is both simple and convenient, but there are several possible sources of error, and it has been found that slight differences in the percentage of moisture found in a given malt will be obtained, depending upon the position of the dish in the water bath, the size of the water bath used, and whether the dish is in actual contact with the water jacket or on the shelf above it.

Differences so obtained are, however, quite small, and for all practical purposes the results are sufficiently accurate. No doubt drying *in vacuo*, at a standard temperature, or out of contact with the air in the presence of such a gas as carbonic acid gas or hydrogen would be more strictly correct, but such methods hardly come within the possibilities of technical working, whilst some part of the moisture may well be in a state of actual combination, and in any case it is known to be extremely difficult to obtain a true moisture percentage from substances containing sugars by direct drying, so that an absolute value is impossible. The essential point is that the method should be a comparative one.

Determinations are best made in duplicate, and results should not differ by more than 0.3 per cent.

## EXTRACT.

This is always expressed in terms of "per 336 lbs.," not in natural weight, because the natural weight of a malt can seldom be satisfactorily determined on a small sample such as is generally available for analysis, and further because most malt is bought and sold at a standard weight of 42 lbs. to the bushel. If, however, the weight per bushel is known, the extract can, of course, be easily so calculated if desired.

A Seck mill is used to grind the malt for the various determinations in malt analysis, and the rollers are adjusted by

| Specific Gravity. | Barrels Gravity. | Extract. | Specific Gravity. | Barrels Gravity. | Extract. |
|-------------------|------------------|----------|-------------------|------------------|----------|
| 1022·5            | 8·1              | 75·6     | 1026·3            | 9·5              | 88·4     |
| ·6                | 8·1              | 75·9     | ·4                | 9·5              | 88·7     |
| ·7                | 8·2              | 76·3     | ·5                | 9·5              | 89·0     |
| ·8                | 8·2              | 76·6     | ·6                | 9·6              | 89·4     |
| ·9                | 8·2              | 76·9     | ·7                | 9·6              | 89·7     |
| 1023·0            | 8·3              | 77·3     | ·8                | 9·6              | 90·0     |
| ·1                | 8·3              | 77·6     | ·9                | 9·7              | 90·4     |
| ·2                | 8·3              | 77·9     | 1027·0            | 9·7              | 90·7     |
| ·3                | 8·4              | 78·3     | ·1                | 9·7              | 91·0     |
| ·4                | 8·4              | 78·6     | ·2                | 9·8              | 91·4     |
| ·5                | 8·5              | 78·9     | ·3                | 9·8              | 91·7     |
| ·6                | 8·5              | 79·3     | ·4                | 9·8              | 92·0     |
| ·7                | 8·5              | 79·6     | ·5                | 9·9              | 92·4     |
| ·8                | 8·6              | 79·9     | ·6                | 9·9              | 92·7     |
| ·9                | 8·6              | 80·3     | ·7                | 9·9              | 93·0     |
| 1024·0            | 8·6              | 80·6     | ·8                | 10·0             | 93·4     |
| ·1                | 8·7              | 81·0     | ·9                | 10·0             | 93·7     |
| ·2                | 8·7              | 81·3     | 1028·0            | 10·0             | 94·1     |
| ·3                | 8·7              | 81·6     | ·1                | 10·1             | 94·4     |
| ·4                | 8·8              | 81·9     | ·2                | 10·1             | 94·7     |
| ·5                | 8·8              | 82·3     | ·3                | 10·2             | 95·1     |
| ·6                | 8·8              | 82·6     | ·4                | 10·2             | 95·4     |
| ·7                | 8·9              | 82·9     | ·5                | 10·3             | 95·7     |
| ·8                | 8·9              | 83·3     | ·6                | 10·3             | 96·1     |
| ·9                | 8·9              | 83·6     | ·7                | 10·3             | 96·4     |
| 1025·0            | 9·0              | 84·0     | ·8                | 10·4             | 96·7     |
| ·1                | 9·0              | 84·3     | ·9                | 10·4             | 97·1     |
| ·2                | 9·1              | 84·7     | 1029·0            | 10·4             | 97·4     |
| ·3                | 9·1              | 85·0     | ·1                | 10·5             | 97·8     |
| ·4                | 9·1              | 85·3     | ·2                | 10·5             | 98·1     |
| ·5                | 9·2              | 85·7     | ·3                | 10·5             | 98·4     |
| ·6                | 9·2              | 86·0     | ·4                | 10·6             | 98·8     |
| ·7                | 9·2              | 86·3     | ·5                | 10·6             | 99·1     |
| ·8                | 9·3              | 86·7     | ·6                | 10·6             | 99·5     |
| ·9                | 9·3              | 87·0     | ·7                | 10·7             | 99·8     |
| 1026·0            | 9·4              | 87·4     | ·8                | 10·7             | 100·1    |
| ·1                | 9·4              | 87·7     | ·9                | 10·7             | 100·5    |
| ·2                | 9·4              | 88·0     | 1030·0            | 10·8             | 100·8    |

means of a feeler gauge so that they are 0·5 mm. apart. The scale pointer of the mill is then usually, but not always, at 25°.

The determination is conducted as follows:—Weigh out rather more than 50 grammes of the malt in a tared metal scoop, and, after first grinding a few corns through the mill to clean it out, grind the whole quantity and collect in the copper beaker, brush the ground malt back again into the tared scoop, taking care that none of the fine mealy dust is lost, and weigh out exactly 50 grammes, removing any excess with a spatula.

Now brush the weighed malt through a funnel into a 515 c.c. flask, which should have a neck sufficiently wide to allow the stem of the funnel to pass well down into the bulb, the diameter of the neck may be about half an inch, and the funnel, preferably made of copper, should have a stem diameter of at least a quarter of an inch, as the ordinary glass funnels have too narrow a stem for the purpose. Run in 400 c.c. of distilled water previously raised to a temperature of 163°, and during the operation keep the flask thoroughly shaken, giving to it a circular movement so as to prevent “balling” and ensure thorough mixing of the malt and water. By this means the actual temperature of the mash in the flask should be approximately 150°, and the original striking heat of the water used must be varied slightly if necessary to obtain this.

The flask is then put into a water bath kept at a temperature of 150°—152° for 50 minutes, after which time the temperature in the outer bath is raised to 165° and the flask well shaken, the actual finishing temperature of the mash being about 158°. The flask and contents are then cooled

down to 60°, made up with ordinary water to the 515 c.c. mark, the whole thoroughly well mixed, filtered into a dry beaker, and the specific gravity taken.\*

The specific gravity of wort is reduced to lbs. per barrel by multiplying by 0.36, and the dilution is at the rate of 9.33 barrels per quarter. Then  $9.33 \times 0.36 = 3.36$ , the factor used.

*Example.*—A wort thus obtained gave a specific gravity of 1029.0°, the excess weight over 1000, namely, 29.0, multiplied by 3.36 = 97.4 brewers' lbs. per standard quarter of malt.

**Seck Mill.**—The Seck mill (see p. 110) is perhaps the best instrument obtainable for getting comparative conditions of grinding in malt analyses, and is now, on the advice of the Malt Analysis Committee of 1906, the one generally used in brewers' laboratories for grinding the malt for the various determinations made in malt analysis. It was originally agreed that the mill should be set at 25°, but it has recently been found that these mills will vary, often very considerably, in the actual distances apart of the rollers, even when set at the same scale of 25°, and as a result different mills will be found to give different fineness of crushing, and so extracts will vary with the mill used.

It is, therefore, essential to use a measure gauge or "feeler," and to adjust the rollers so that they will allow a 0.5 mm. gauge to pass through when the rollers are set ready for the determination, and it should also be remembered that continued use of the mill will to some extent alter the roller distance, and the gauge test ought frequently to be tried. Further, it has been found that the rollers when so set are not always uniform in their distance apart throughout the whole length, and these, if they are not so, must be readjusted.

The gauge test should be made at several places along the length of the rolls with handle situated at each 45°—four positions.†

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\* See p. 7.

† Where a mill has been in use for any considerable time, the rollers may become somewhat worn, in which case the standard of 0.5 mm. distance between the rollers for the setting of the mill is not confirmatory that the old rollers will grind to give the same extract from the sample of malt as will new rollers set at the same distance apart.

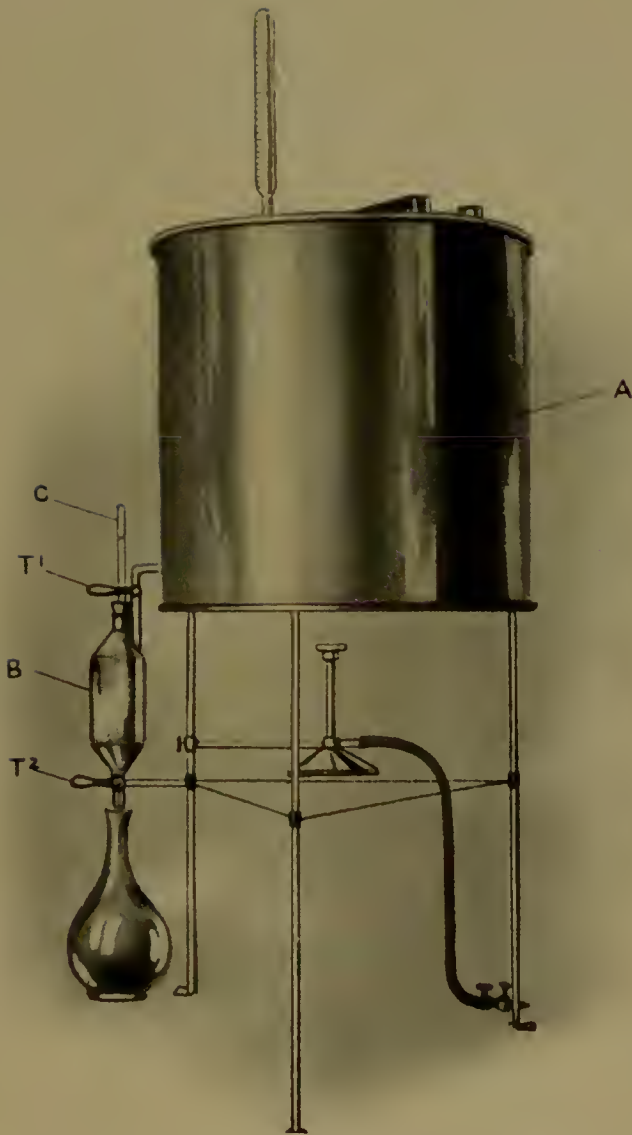
**Distilled Water.**—It may be found more convenient to use ordinary boiled tap water in place of distilled for the estimation of extract, and normal calcium carbonate water if so used will make no difference in the results. Such a laboratory supply should be boiled for 20 minutes, allowed to settle, if necessary filtered, and then brought back to the desired temperature for use.



SECK MILL.

**Extract Bath.**—Where a number of extracts are to be determined together, it is more convenient to use a special heating bath for mashing instead of using beakers for measuring out and heating each 400 c.c. of water required. The figure shows how this is effected.

The copper bath A contains the water whose temperature is to be raised to  $163^{\circ}$ . B is a cylindrical copper vessel of capacity 400 c.c. at  $60^{\circ}$  when filled with water up to the mark C on the glass tube at  $163^{\circ}$  F. The water can first be run into the cylinder by opening the tap T 1, closing this when the water reaches the mark C, and at once turning the tap T 2, running directly into the flask containing the malt. By using a bath of this sort, a great many mashes can be accurately and quickly made.



MALT EXTRACT BATH.

For determining a number of Hot Extracts of Malt.





**515 Flask.**—A 515 c.c. flask is used, as it is assumed to represent the bulk occupied by 500 c.c. of wort together with the grains from 50 grammes of malt. The amount of "grains" in a malt is not always the same, for, with thin, husky malts, the insoluble matter is greater than with thin-skinned and heavy home barleys. No very great error, however, would be introduced by the slight difference in the bulk occupied.

The above method is modified from one originally suggested by Heron,\* and has now replaced that described in the last edition of this book, which the author intended should compare as closely as possible with the practical determination of extract obtained in the brewery.

That just described has now been in use in our laboratory for a number of years, and has the great advantage of being simple and comparatively easy to carry out with accuracy. If a duplicate determination of an extract is made, the specific gravity of each of the resulting worts should not be found to differ by more than  $0.2^{\circ}$ .

The Malt Analysis Committee of 1906 recommend some slight variations in the method above described, and suggest the use of a beaker in place of the 515 c.c. flask for making the mash, though at the same time they recommend the flask as an alternative. When using a beaker, the malt is ground in the usual way and mashed in the beaker (presumably standing in a water bath) with 360 c.c. of distilled water previously heated to  $154^{\circ}$ . The mash is allowed to stand for 50 minutes and stirred at intervals of 10 minutes, between which times it is covered with a clock glass. The temperature of the mash is finally raised to  $158^{\circ}$  and

\* *Journ. Chem. Soc.*, 1888.

afterwards cooled back to 60°, washed into a 515 c.c. flask, afterwards proceeding as already described.

## COLOUR OF WORT.

This is registered by means of a most simple yet ingenious instrument, known as Lovibond's Tintometer. In this apparatus the liquid to be examined is placed in a cell with glass ends and its colour imitated by means of one or more coloured glasses of known value. The sum of the values of the glasses used gives the tintorial power of the liquid under examination. In order to facilitate such a comparison, the liquid and standard glasses are viewed through a tube against a background of white porcelain or dull white paper.

A small portion of wort is filtered through a dry filter paper (in which a little Kieselguhr has been placed to assist filtration) into a dry beaker, and the tintometer value ascertained in a 1-inch cell and expressed on a 20-lb. unboiled wort.

When the colour of a wort is as pale as 3° (10 per cent. solution) it will be found more accurate to use a 2-inch cell.

The Malt Analysis Committee recommend the expression of colour as degrees on a 10 per cent. solution—1-inch cell, but in many ways it is more convenient to express it on a 20-lb. wort, and although, of course, the colours of wort do not vary in strict arithmetical proportion with the gravity, the error introduced is a very small one.

*Example :—*

Wort examined had a specific gravity of  $1027.0 = 9.7$  lbs. per barrel.

This in 1-inch cell gave  $7.5^\circ$  on Lovibond scale.

Thus  $9.7 : 20 :: 7.5 = 15.5$ , the tintometer value of a 20-lb. unboiled wort.

The standard glasses used are those known as the 52 series, and these are generally found satisfactory for the determination of colour in malt worts.

Some analysts prefer to use a red 50 series and yellow 52 series together, and build them up in such a way as to more closely imitate the actual colour of the solution, but with malt worts this has not been found a desirable method of procedure, and it is only where the malt has been considerably scorched and slightly caramelised that any material amount of red shade is found in the extracted solution.

It is advisable to take readings each side of the tintometer by reversing the glasses and the cell, and there is very little difficulty in taking these colours with a good supply of daylight available, but where, as often happens, this is not the case, results made by different observers will not agree, and there is no question that it is very difficult to get good results with artificial light. As far as possible the white porcelain or paper screen should be brightly illuminated.

Many proposals have been suggested for overcoming the difficulty, but no standard light has at present been used which is entirely satisfactory.

It should be mentioned also that it is sometimes impossible for the same observer to get a similar colour reading from *different extracts* of the same malt, and this particularly with high dried malts where a percentage of the corns are coloured or scorched on the kiln. A difference of as much as  $3^\circ$  or  $4^\circ$  on a 20-lb. wort is quite possible, and in such cases the average reading must be taken.

Recent papers dealing with the question of colour in malts may be referred to :—

Baker and Hulton, *Journ. Inst. Brew.*, 1906, pp. 302–309.

J. W. Lovibond, *Journ. Inst. Brew.*, 1908, pp. 2–7.

## TOTAL MATTERS SOLUBLE IN COLD WATER.

Two methods may be used for the determination, and the one now to be described is that used in our laboratory, and is as follows :—

Make a 5 per cent. cold-water extract of the malt.\* Weigh out rather more than 10 grammes of the malt, and after passing a little through the Seck mill, set with the rollers 0.5 mm. apart, to clean it, grind the malt and weigh out exactly 10 grammes.

Transfer by brushing through a funnel, similar to that used for hot extracts, into a clean bottle (an ordinary pint bottle will do perfectly well), and add 200 c.c. of distilled water at 60° F. Close the bottle with a clean indiarubber stopper, thoroughly mix the malt and water by continual shaking, taking care that no malt is left caked to the bottom, and allow to stand for three hours. In place of a bottle a large beaker can equally well be used, and the malt and water thoroughly mixed by means of a glass rod. Do not allow the temperature to rise above 60° during the time of stand.†

\* Where the soluble nitrogen is to be determined the method of procedure is slightly different, and the cold extract of malt is then made as described on p. 118, and is in that case a 10 per cent. solution.

† Morris has shown (*Journ. Inst. Brew.*, 1896, p. 224) that under the above conditions of malt extraction there is no appreciable attacking of the starch cells, but more recently Ling (*Journ. Inst. Brew.*, 1898, vol. 9, p. 189) shows that some hydrolysis takes place, with production of maltose, and suggests extraction in alkaline solution. See alternative method, p. 116.

Matters Soluble in Cold Water, 5 per cent. Solution of  
Malt.

| Per cent. on Malt. |      | Per cent. on Malt. |      |
|--------------------|------|--------------------|------|
| 1002·50            | 12·9 | 1003·85            | 19·9 |
| ·55                | 13·2 | ·90                | 20·2 |
| ·60                | 13·4 | ·95                | 20·4 |
| ·65                | 13·7 | 1004·00            | 20·7 |
| ·70                | 14·0 | ·05                | 20·8 |
| ·75                | 14·2 | ·10                | 21·2 |
| ·80                | 14·5 | ·15                | 21·5 |
| ·85                | 14·7 | ·20                | 21·7 |
| ·90                | 15·0 | ·25                | 22·0 |
| ·95                | 15·2 | ·30                | 22·2 |
| 1003·00            | 15·5 | ·35                | 22·5 |
| ·05                | 15·8 | ·40                | 22·8 |
| ·10                | 16·0 | ·45                | 23·0 |
| ·15                | 16·3 | ·50                | 23·3 |
| ·20                | 16·5 | ·55                | 23·5 |
| ·25                | 16·8 | ·60                | 23·8 |
| ·30                | 17·1 | ·65                | 24·0 |
| ·35                | 17·3 | ·70                | 24·3 |
| ·40                | 17·6 | ·75                | 24·6 |
| ·45                | 17·8 | ·80                | 24·8 |
| ·50                | 18·1 | ·85                | 25·1 |
| ·55                | 18·3 | ·90                | 25·3 |
| ·60                | 18·6 | ·95                | 25·6 |
| ·65                | 18·9 | 1005·00            | 25·9 |
| ·70                | 19·1 | ·05                | 26·1 |
| ·75                | 19·4 | ·10                | 26·4 |
| ·80                | 19·6 | ·15                | 26·6 |

Matters Soluble in Cold Water—*continued.*

| Per cent. on Malt. |      | Per cent. on Malt. |      |
|--------------------|------|--------------------|------|
| 1005·20            | 26·9 | 1005·55            | 28·7 |
| ·25                | 27·2 | ·60                | 29·0 |
| ·30                | 27·4 | ·65                | 29·2 |
| ·35                | 27·6 | ·70                | 29·5 |
| ·40                | 28·0 | ·75                | 29·7 |
| ·45                | 28·2 | ·80                | 30·0 |
| ·50                | 28·5 |                    |      |

Shake vigorously at the end of the three hours and filter through a dry paper into a dry beaker. The extract should be returned once or twice through the paper until the filtrate comes through bright. Take the specific gravity and divide the excess weight over 1,000 by the factor 3·86,\* which, multiplied by 20, gives the grammes of dry solids per 100 c.c., calculated on 100 parts of malt, the result being the total matters soluble in cold water per cent.

*Example :—*

Sp. gr. was found to be 1003·9°.

Then  $3·9 \div 3·86 = 1·010$  dry solids per 100 c.c. of solution, and  $1·010 \times 20 = 20·20$  dry solids per cent. of malt or “matters soluble in cold water.”

The diastatic power can also be determined from this solution, as described on p. 123.

*A second method* for extracting the total soluble matters in cold water is due to A. R. Ling,† and depends on the addition of an alkali to the water, so preventing any possible

\* For explanation of factor, see p. 101.

† *Journ. Inst. Brew.*, 1898, vol. 9, p. 189. A. R. Ling proposed potash or soda as the alkali.

diastatic action taking place during extraction. The method is recommended by the Malt Analysis Committee and is as follows :—

Extract 25 grammes of the ground malt with 250 c.c. of distilled water containing 20 c.c. of N/10 ammonia, for three hours at 70°, stirring three or four times during this period. After filtering, the specific gravity of the bright filtrate is taken, and the total matters soluble in cold water are obtained from the gravity exactly as previously described.

The method works quite satisfactorily, though filtration is decidedly slow. The figures obtained by this method for matters soluble in cold water have no precise relation to those obtained when extracting the malt as previously described with untreated distilled water, but as a rule the ammonia method gives from 1 to 2 per cent. lower figures. The following table will illustrate this :—The figures give the percentages of matters soluble in cold water as extracted by water alone in Column “A,” and water to which ammonia is also added in Column “B.”

|  | “ A.”     | “ B.”     |
|--|-----------|-----------|
|  | Per cent. | Per cent. |
| 1. A sound and tender malt ... ..        | 18·1      | 17·3      |
| 2. A fairly tender English ... ..        | 18·6      | 17·3      |
| 3. Undercured English ... ..             | 19·6      | 17·6      |
| 4. Forced English ... ..                 | 22·3      | 20·7      |
| 5. Tender Foreign ... ..                 | 16·8      | 16·0      |
| 6. A steely high-coloured Foreign ... .. | 18·3      | 17·6      |



**Determination of Total Soluble Nitrogenous Bodies.**—Where a direct estimation of these substances is required, proceed as follows :—

Make a 10 per cent. extract by grinding rather more than 50 grammes of the malt through a Seck mill, previously cleaned as before by crushing a little of the malt through the rollers (0·5 mm. apart), and weigh out exactly 50 grammes of the ground malt.

Extract with 500 c.c. of distilled water for three hours at 60°. Filter through a dry paper and afterwards measure out 300 c.c. of the bright filtrate by first rinsing a 300 c.c. graduated flask with a little of the extract, and then carefully making up to the mark at 60°. Pour the wort into a beaker, putting the flask aside for making up to volume again presently, and boil vigorously for five or ten minutes. By this means all the coagulable nitrogen is thrown out of solution, the extract is then cooled back to 60° and transferred again to the 300 c.c. flask previously used, and made up to the mark with distilled water, thoroughly mixed and filtered.

The specific gravity of the boiled cold extract, if now taken, should be only very slightly less than that obtained for the unboiled extract—the amount of nitrogen thrown out in boiling making but slight appreciable difference in the gravity, and the total matters soluble in cold water can, if required, be calculated from the specific gravity so obtained, remembering that it is a 10 per cent. solution.

The nitrogen is determined by Kjeldahl's process as follows :—

Place 50 c.c. of the cold-water extract in a small boiling

flask (8 oz.), and evaporate down till practically dry, taking care not to char the residue. Add 20 c.c. of strong sulphuric acid, and 10 grammes of potassium sulphate. Warm gently at first, and when the contents of the flask have broken down into a fluid state, boil vigorously over a gas flame. Place a small funnel covered with a watch glass in the mouth of the flask to condense the acid fumes given off, for which reason also the boiling should be done in a special "stink" cupboard. Continue the boiling until the solution becomes clear, and either colourless or of only a faint yellow tinge. This should take place in about two hours. Now allow to cool. Then wash into a distilling flask, special forms of which can be obtained for the purpose, with about 200 c.c. of water. Be careful in at first adding the water to the strong acid. Wash the whole into the flask, then add 75 c.c. of sodium hydrate solution (sp. gr. 1500), and a few pieces of pumice stone to prevent bumping, or short pieces of drawn-out capillary tube, attach the flask to condenser, and distil. Collect the distillate in a wide-mouthed flask in which 20 c.c. of decinormal sulphuric acid has been previously placed, the flask being connected with the condenser by means of an adapter, the end of which must dip beneath the surface of the acid.

Distil over 120 to 150 c.c., by which time the whole of the ammonia produced by decomposition of the nitrogenous bodies in the solution will have passed over, and have been dissolved in and neutralised by the acid. Now add to the distillate a few drops of methyl orange, as an indicator, and titrate with decinormal alkali. The alkali is run in

until the pink colour of the solution just changes to yellow, as methyl orange is pink with acids, yellow with alkalies. Note number of cubic centimetres required. This, deducted from the quantity of acid placed in flask—20 c.c.—gives the number of cubic centimetres of acid neutralised by the ammonia due to the protein\* in the cold water extract. Each cubic centimetre of decinormal acid equals 0.0017 ammonia, or 0.0014 nitrogen, but it is more useful for our purpose to express the nitrogen in the form of protein, although the actual percentage of nitrogen in the various protein compounds dissolved out from malt is not known with precise accuracy—they are generally considered, however, to contain on the average 15.7 per cent. of nitrogen, so that the factor 6.36 ( $100/15.7$ ) is commonly used. Or we may immediately multiply the number of cubic centimetres neutralised by the factor 0.009 (0.0014 multiplied by 6.36), and this will give the protein directly.

Some correction is necessary for the nitrogen in the reagents used. This may be arrived at by taking through a blank experiment with the acid and the sodium hydrate solution, etc., used, and deducting the amount so found from the total. The correction is generally equal to between 0.6 and 1 c.c. of decinormal acid.

\* Hitherto known as "Albumen" and "Albuminoids," but these terms have in recent years been more usefully replaced by "Protein," and "Proteins." (See Chapter II, Part I.)

*Example :—*

In titrating the distillate with N/10 alkali, it was found that 5.4 c.c. was required to neutralise the acid.

Therefore,  $20 - 5.4 = 14.6$  c.c. N/10 acid neutralised by the distillate.

From this must be deducted the correction found in the blank experiment, in this case equal to 0.6 c.c. of acid :—

$14.6 - 0.6 = 14$  c.c. N/10 acid neutralised by the ammonia derived from the proteins, and

$14.0 \times 0.009 \times 2 = 0.252$  protein in 100 c.c. of cold-water extract.

This multiplied by 10 gives 2.52 per cent. on the malt.

**Determination of Mineral Matter.**—This is not, as a rule, necessary, but if it is desired to estimate the exact percentage, 25 c.c. of the cold extract (5 per cent. solution) are measured into a platinum dish by means of a pipette and boiled down as low as possible. This must be done cautiously, or there may be some spurting and consequent loss, as the solution generally froths on heating. When nearly evaporated add another 25 c.c. of wort, making 50 c.c. in all, and boil down as before. When apparently dry, very cautiously apply flame until the carbonised ash has ceased to smoke or give off fumes. Now cover loosely with a piece of platinum foil, and continue ignition until the ash is grey or white; cool under dessicator and weigh. The increase of weight is due to ash from 50 c.c. of cold-water extract. Calculate to percentage on malt.

*Example :—*

50 c.c. of 5 per cent. cold-water extract used—

|              |     |     |     | Grammes. |
|--------------|-----|-----|-----|----------|
| Dish and ash | ... | ... | ... | 36·460   |
| Dish alone   | ... | ... | ... | 36·406   |
|              |     |     |     | -----    |
|              |     |     |     | 0·054    |

Now  $0·054 \times 2 = 0·108$  per cent. on solution, and  
 $0·108 \times 10 = 1·08$  per cent. mineral matter on malt.

**Ready-Formed Soluble Carbohydrates.**—Where an estimation of soluble ash and soluble nitrogenous bodies has been made, the ready-formed soluble carbohydrates are obtained by deducting the sum of these two, together with the acidity (see p. 129) from the total matters soluble in cold water.

It is not always possible to make a detailed analysis of the cold-water extract in the way described, and in this case it is usual, and is so advised by the Malt Analysis Committee, 1906, to obtain the ready-formed soluble carbohydrates by deducting 4 per cent. from the total matters soluble in cold water as obtained on p. 114.

The ready-formed soluble carbohydrates are difficult to determine directly, and they include several sugars, certainly cane and invert sugar, which are not, of course, in any way harmful to a good malt.

As of helpful value in the interpretation of malt analysis, the total matters soluble in cold water should certainly be estimated,\* and in certain cases also the soluble nitrogenous

\* But see Interpretation, p. 138.

bodies, although this latter determination takes considerable time.

It is rarely necessary to actually determine the percentage of mineral matter, as this figure varies only very slightly with different malts and is approximately 1.1 per cent.

### DIASTATIC POWER.\*

The correct determination of this figure is of much importance in a malt analysis, because not only does the figure indicate the diastatic activity of the malt, but affords also, in conjunction with other data, valuable information as to the manner in which the malt has been made. Any attempt to separate the diastase quantitatively would be practically futile, and the diastatic power, and not its precise amount, is, therefore, always determined.

The best method is undoubtedly that of Lintner, which has now almost entirely displaced older and less satisfactory processes. It is founded on the fact, established by Kjeldahl, that the activity of diastase is measurable by the amount of maltose produced when it reacts upon starch, provided the starch be in a definite condition, and that the maltose does not exceed in amount half the weight of the conversion products of the starch.

For the estimation a 5 per cent. cold extract of malt is used. This will have been obtained in the determination of total matters soluble in cold water, but if the diastatic power *only* is to be determined then 25 grammes of malt ground through a Seck mill at 25° (gauge measure 0.5 mm.) may

\* See a recent paper by J. O'Sullivan "On an Improvement in the Method of Malt Analysis," *Journ. Inst. Brew.*, 1911, pp. 35-48.



be digested with 500 c.c. of distilled water at 60° F. for 3 hours, keeping the solution in a cool place so that the temperature does not materially rise, and at the end of this time filtered and a bright filtrate obtained.

Now prepare a 2 per cent. solution of soluble starch. Weigh out 2 grammes and wash by placing it in a large beaker, filling up with tap water and allowing it to settle. Decant off and again fill the beaker with water and pour off. This washing is most desirable, despite the fact that the starch may have been prepared with every care. Now work up the starch into a cream with a little water, run this cream into 100 c.c. of boiling pure distilled water, just raise again to the boil and then cool back to 70°. It is most important that the water used for making up the starch solution should be perfectly pure, and it is safer to distil the ordinary tap water twice, the second time adding a little permanganate of potash and rejecting the first portion of the distillate. The water should be perfectly neutral to methyl orange and be free from nitrites.

Now take five carefully cleaned test-tubes and add to each 10 c.c. of the freshly prepared starch solution at 70° F. For all average malts likely to have a diastase of 28°—42° add the following amounts of the 5 per cent. malt extract to each of the five test-tubes respectively, 0.24, 0.30, 0.34, 0.40, and 0.50 c.c., using for the purpose a delivery pipette tube of narrow bore, graduated to 1/50th c.c. Mix contents of the tubes by careful but thorough shaking and place in a suitable stand immersed in a bath of water kept at 70° F. Should the malt be suspected of being high dried and so likely to have a lower diastase than 28°, the following



amounts are best added to the five tubes in place of those already given, namely, 0·30, 0·40, 0·50, 0·60, and 0·70 c.c. of the cold extract respectively, whilst for high diastatic, including vinegar, malts, the best range of tubes would be a series containing 0·08, 0·10, 0·12, 0·14, 0·16 c.c. respectively, but with such malts it is more accurate to dilute the original 5 per cent. solution to one of 2·5 per cent. and use a series of tubes with 0·10, 0·14, 0·20, 0·24, 0·30 c.c.

Allow to stand for exactly one hour, keeping the temperature constant at 70° throughout this time. Then add to each tube 5 c.c. of Fehling's solution,\* which may be run in from a burette. Mix thoroughly, and place the tubes in boiling water for 10 minutes. Then place the test-tubes in a stand, and allow to settle. Note the tube in which the blue colour of the Fehling's solution is just destroyed. The tube in which this is observed is taken as the reading for the calculation of the diastatic power. It often happens that there is a blue colour in one tube, and the next is "overdone," that is, the liquid is yellow. In this case, the mean between the two is taken. Thus the test-tube containing 0·30 c.c. of malt solution may be blue, that containing 0·34 c.c. may be distinctly "overdone." In this case the reading of 0·32 c.c. will be taken.

It is best to take readings when the tubes have been allowed to stand for a short time, but if they are left, say, overnight, the colour of the Fehling's solution tends to increase slightly, so that rather lower diastatic powers would, in this case, be obtained than if the tubes were read off at once.

\* For preparation see p. 188.

## Diastatic Power.—Lintner's Method.

|        | °   |         | °  |
|--------|-----|---------|----|
| ·10... | 100 | ·36...  | 28 |
| ·11... | 91  | ·37...  | 27 |
| ·12... | 83  | ·38...  | 26 |
| ·13... | 77  | ·40...  | 25 |
| ·14... | 71  | ·42...  | 24 |
| ·15... | 67  | ·43...  | 23 |
| ·16... | 62  | ·45...  | 22 |
| ·17... | 59  | ·47...  | 21 |
| ·18... | 55  | ·50...  | 20 |
| ·19... | 52  | ·52...  | 19 |
| ·20... | 50  | ·55...  | 18 |
| ·21... | 48  | ·57...  | 18 |
| ·22... | 45  | ·60...  | 17 |
| ·23... | 43  | ·62...  | 16 |
| ·24... | 42  | ·65...  | 15 |
| ·25... | 40  | ·67...  | 15 |
| ·26... | 38  | ·70...  | 14 |
| ·27... | 37  | ·72...  | 14 |
| ·28... | 36  | ·75...  | 13 |
| ·29... | 34  | ·77...  | 13 |
| ·30... | 33  | ·80...  | 13 |
| ·31... | 32  | ·85...  | 12 |
| ·32... | 31  | ·90...  | 11 |
| ·33... | 30  | ·95...  | 10 |
| ·34... | 30  | 1·00... | 10 |
| ·35... | 29  |         |    |

Lintner's standard for malt is as follows :—The diastatic power of a malt is to be taken as 100°, when 0.1 c.c. of 5 per cent. solution of malt treated as described just reduces 5 c.c. of Fehling.

*Example :—*

Reading taken on second tube = 0.30 c.c. of malt solution required to decompose the 5 c.c. of Fehling.

$$\text{Then } 0.3 : 100 :: 0.1 = 33.3^\circ.$$

The cold extract of malt contains a small amount of reducing sugars, which can be ascertained by direct experiment; but it is not found desirable to make any correction for this.

An *alternative method* devised by A. R. Ling\* may be used for the determination of diastase, and is described in the report of the Malt Analysis Committee of 1906.

Twenty-five grammes of ground malt are to be extracted with 500 c.c. of distilled water (see p. 123) for three hours at 70° F. and filtered bright, stirring well every half hour. A portion of the filtrate (3 c.c.) is allowed to act on 100 c.c. of a 2 per cent. solution of soluble starch (see p. 415) at 70° F. for an hour in a 200 c.c. flask. N/10 caustic alkali (10 c.c.) is then added in order to stop further diastatic action, the liquid cooled to 60° F., made up to 200 c.c. with distilled water at the same temperature, well shaken, and titrated against 5 c.c. portions of Fehling's solution, using ferrous thiocyanate as indicator.

\* *Analyst*, 1905, vol. 30, p. 182.

The titration is carried out as follows :—

Five cubic centimetres of Fehling's solution are accurately measured into a 150 c.c. boiling flask, and raised to boiling over a small naked Bunsen flame. The converted starch solution is added from a burette, in small quantities at first of about 5 c.c., the mixture being kept rotated and boiled after each addition until reduction of the copper is complete, which is ascertained by rapidly withdrawing a drop of the liquid by a glass rod, and bringing it at once in contact with a drop of the indicator on a porcelain or opal slab.

When all the cupric salt has been reduced the indicator will cease to show a red colour.

This indicator is perhaps best used as a guide only, and in any case a very little experience will soon enable the finishing point of the reaction to be determined, seen by the peculiar boil and appearance of small bubbles.

It is a good plan to decant off the contents of the boiling flask into a test tube after the copper has been reduced, and this when settled will confirm the result obtained. The liquid should be colourless or only very slightly yellow.

The preparation of Fehling's solution and of the indicator is dealt with on pp. 188 and 421.

The test-tube method previously described has some advantages over the flask method, in that it enables a better check to be made on the working, and the satisfactory determination of diastase requires very great care and attention to detail, and is not at all easy. Both the above processes give practically identical results.

## ACIDITY.

This may be determined by treating 50 grammes of ground malt with 200 c.c. of cold distilled water, allowing to stand one hour with occasional stirring. The whole may then be titrated with decinormal soda, using litmus paper as an indicator. Preferably, however, the liquid is passed through a dry filter paper, and 100 c.c. titrated, the same indicator being used. Each cubic centimetre of alkali equals 0.009 gramme of lactic acid.

Another plan is to titrate a portion of the wort as obtained in the mashing operation; but this can only be done if the mash has been made with distilled water, otherwise much of the acidity will have been neutralised by such carbonates as may remain in the boiled water.

The acidity of malt is due not only to lactic acid, but also to other organic acids and acid phosphates, and Fernbach\* recommends the use of phenolphthalein as an indicator, the amount of alkali required then being the measure of the acidity due to the acid phosphates, together with free acids such as lactic, but good results have not been obtained by this method, and no really satisfactory one for the determination of acidity in a malt has yet been devised.

### *Example:—*

Fifty grammes of malt, after extraction with cold water, required 7 c.c. of decinormal alkali, using litmus paper as an indicator. Then  $7 \times 0.009 = 0.063$  acid in 50 grammes of malt, expressed as lactic acid, or 0.126 per cent.

\* *Journ. Inst. Brew.*, 1896.

## SACCHARIFICATION.

This test measures the speed with which the diastase of the malt saccharifies the starch. It is not so much the measure of the diastase of the malt, as of the condition of the starch itself. A tender malt will almost always saccharify rapidly, a steely malt slowly.

The test is thus performed :—

Ten grammes of malt are fairly coarsely ground, and mashed in a beaker with 100 c.c. of water at 155° F. Keep in a bath at 150°. At the end of 20 minutes stir, allow to roughly settle, and withdraw a few cubic centimetres of the wort. Place on a porcelain plate, or cover, and test for starch by addition of iodine solution. If starch is present, the test is repeated in fresh portions of the wort, abstracted at further intervals of five minutes, until conversion is complete. The time required for complete saccharification is noted. It will often happen, particularly with steely malts, that starch nearly disappears after, say, 30 minutes, but that a small quantity persistently remains for a considerable further time.

**Iodine Test for Starch.**—The method of testing for starch in worts is very simple, and with last runnings very necessary; but it often happens that the brewer fails to find starch when it is really present. It must be remembered that iodine forms red or brown colorations with maltose and the dextrins. Soluble starch possesses, however, a greater affinity for iodine than the dextrins, so that a trace of starch may be detected by adding only a very small quantity of iodine. If a large quantity is at once added, the deep reddish-brown colour due to dextrins will generally mask the presence of traces of soluble starch.

The wort to be tested *must be cold*, as the characteristic coloration

is discharged by heat, though it reappears on cooling if the heat has not been sufficiently prolonged to volatilise the iodine.

It is an excellent plan to place a few drops of the wort to be tested on a flat evaporation slab (the upturned bottom of a saucer answers well). Then drop on to the plate, so as not to actually touch the wort, a little iodine solution; the plate is then tilted so that the iodine solution and wort meet, in which case, if starch is present, a blue coloration will generally appear at the point where the iodine touches the wort, or a little beyond it.

The iodine solution, as purchased from a chemist's shop, is an alcoholic tincture. An aqueous solution of iodine in iodide of potassium is to be preferred.

## PHYSICAL EXAMINATION.

Useful as are analytical methods of malt valuation, these must be supplemented by a careful physical examination. The general appearance of the malt should first be noted, the extent of acrospire growth, the absence or presence of mould (noting whether red or blue), the presence of damaged or half corns, or those attacked by weevil. Do not pay too much attention to the "brightness" of the sample. The brightest malts are sometimes the worst made, and malts made from kiln-dried barleys are generally of poorer colour than those made direct, whilst thorough withering generally also means loss of colour. Note the evenness or otherwise of the colour of the coombs or rootlets. The proportion of sinking corns (*i.e.*, corns which sink when thrown into water) may be registered, but this is of little use unless the sinking corns are themselves examined to ascertain what proportion of them are dead, for whereas some fully malted corns may sink, those which are dead are sure to do so.

Then chew some of the malt and note its flavour. Do



not merely bite a few corns—chew down a mouthful, and any unpleasant “back” flavour will be then detected. Malts which taste very sweet are suspicious. Undercured malts generally have, not merely a “pasty” flavour, but a certain peculiar bitterness also, only to be detected *after* the malt has been well chewed down. Take careful note of these observations, and compare them with the analytical results.

The corns should be cut with a farinator, and an examination made of the endosperm, particularly noting the distribution of colour over the cut corns and the friability of the contents.

## INTERPRETATION OF MALT ANALYSIS.

In forming an opinion of the value of a malt, it is necessary to base it not solely on either a physical or chemical examination, but on a comparison of both, and on no one figure in the chemical determinations, but upon a careful consideration of all these results taken together, with due regard to the particular purposes for which the malt is required.

Satisfactory malt is at the basis of sound brewing, and a correct interpretation of analysis is of the utmost importance.

**Mould.**—Mould is more an indication of bad flooring conditions or unsatisfactory barley rather than harmful in itself. Red mould is less often found than blue, but is considered more dangerous.

According to Windisch mouldy malts are objectionable, as they secrete a peptonising enzyme, thereby increasing the non-coagulable nitrogenous bodies in the wort.

It is generally found that if the temperature of the growing piece is allowed to rise abnormally, particularly in the early stages, as in the couch, for the purpose of hastening growth, mould is certain to show afterwards. At the same time, it is always difficult to prevent mould growth where there is any quantity of dead or damaged corn, and once this appears it will very quickly spread. For this reason maltsters are sometimes obliged to load their piece sooner than they would otherwise have done.

**Growth.**—The growth of acrospire should be regular, and sufficiently up the corn to ensure complete modification of the endosperm contents. This need not necessarily mean as much as three-quarters or full length of corn, and a mellow, kindly barley will often show an acrospire length of not more than two-thirds and yet be thoroughly well modified.

Poorer quality barleys, or barleys with less vitality, often tend to die off before modification is completed, unless supplied with sufficient sprinkling water, and such barleys, when well modified, usually show rather longer acrospire.

Overshot corns are generally, but not always, found when growth has been forced, and, if so, the general growth will be found to be irregular.

An acrospire which has “broadened out” or “ridged up” in the corns suggests forcing, and this indication will be understood when it is remembered that the acrospire or germ is the chief seat of the soluble nitrogenous bodies.

Slow, cool growth in germination, with acrospire regularly but not too far grown up, yet with complete modification of the endosperm cells, will give the best results for English beers. Too vigorous a growth with high temperatures and excessive sprinkling will not only over-modify and spoil a malt but will result in excessive respiration and loss of yield.

Some maltsters prefer not to sprinkle their malts at all, relying on

a longer steep, but not all barleys will allow of this treatment, although the loss by respiration, etc., is reduced by so doing, and the yield obtained proportionately greater.

The amount of sprinkling water used may easily be too large, but enough must be used so as not to endanger the vitality of the growing barley, and to allow the piece to grow well up to the withering stage. Artificial mellowing by high withering temperatures or stewing on the kiln will not replace weak and incompleting growth and modification on the floors.

### Moisture.

The moisture of malt may be as low as 0·5 per cent. when fresh from kiln, but it rapidly increases to about 1·5 per cent., and in a properly stored malt will not exceed 2·5 or 3 per cent. The practice of storing malt in large heaps in open lofts, often with very unsatisfactory exclusion of moist outside air, is responsible for many of the difficulties of slack malt. Fortunately, however, the practice of storing in metal-lined bins is increasing, and malt may be thus kept without appreciable change for long periods. If the moisture exceeds 3·5 per cent., the malt certainly ought to be redried before use. A high moisture percentage is often—not always—accompanied by a high acidity, and slack malts are certainly dangerous, undoubtedly interfering with fermentation and lowering the stability of the beer. Malts which have been originally somewhat lightly cured generally show less soundness when they are slack than malts originally well cured. Redrying improves the malt, but deterioration due to slackness is never entirely got rid of.

### Extract.

The maximum extract value of a malt will depend upon

the starch-content and the completeness of its modification into a form readily acted upon by the diastase ferments.

Size of grain is not by any means necessarily in proportion to the extract. The bold, often fine looking, Brewing Chilian malts will generally give lower extracts than the smaller looking Brewing Californian. And similarly, many of the small Eastern County malts will give surprisingly good extracts, and some heavy Yorkshire types surprisingly poor ones—depending, of course, largely on the skin. No more serious economical mistake could be made than that of judging the brewing value of a malt solely upon its extract, and the quality of such extract as determined by the general analysis must also be taken into careful consideration.

### Curing.

It is not easy to classify and define flavour and curing, and experience alone can lead to a sound judgment on such matters. With flavour there are such infinite gradations that no hard and fast rule can be laid down; for instance, in some seasons, malts “take the fire” much more readily than in others, so that it is possible to get far more flavour into the material. Again, curing and flavour are not interchangeable terms. Whilst a sound malt will not be raw in flavour, yet such a malt may and does have many different degrees of flavour. Nor can flavour be judged by the tintorial power of its wort, for it is not always the malts which yield the darkest coloured worts which are the richest in flavour, yet there ought always to be some correspondence between the two. Thus, a malt giving a wort

of a colour of  $13^{\circ}$  ought to possess a better and "warmer" flavour than one of  $9^{\circ}$ . But malts which are hurriedly dried will often have much colour, and yet be of poor flavour, and a delicate palate should be easily able to detect under-curing.

It is not desirable, nor, as a rule, is it necessary, to use a malt which has been cured to give a wort of colour paler than  $7.5$  tintometer degrees, expressed on a 20-lb. unboiled wort, although it is quite possible for malt to have been safely cured and yet give a colour of only  $6^{\circ}$  or even as low as  $5.5^{\circ}$ . Malts of this description have occasionally been examined in our laboratory which have been passed as safely cured. This is not, however, the rule, and there are few kilns so constructed as will enable such pale curing to result in a really sound malt.

Where pale stock ales are produced, it is nearly always essential to have a malt which gives a very pale wort, but for the general run of pale ales a malt with a colour of  $8^{\circ}$ – $9^{\circ}$  should be perfectly suitable, and in some districts these beers can be brewed from malts with a colour of  $10^{\circ}$ – $12^{\circ}$ , and where permissible the extra warmth of flavour so obtained is an advantage, and whilst the demand for pale beers is probably increasing, yet there is now less excuse for undercuring than formerly, for it is easy, by the use of substitutes—sugar, raw or prepared grain—to obtain a wort of the necessary paleness, even when a well-cured malt is used.

It will be noticed that barleys which have been damaged or show broken skins are very difficult to cure so as to give a pale coloured wort, and, although the bulk of the

corns from such material may be quite pale cured, the split corns colour readily on the kiln, and increase the average colour of the whole wort when the malt is mashed. This is particularly noticeable when barleys are harvested in a wet condition, and one of the disadvantages of subsequent kiln drying is that it tends to split the skins.

It is most important that a malt should be cured evenly and completely throughout the whole of the grain, and care should be taken to ascertain if this is the case, and that the colour given by the wort fairly represents the entire malt and is not an average obtained from corns, some possibly very pale and unsafely cured, others more or less scorched. Malts of this class are equally unsatisfactory whether obtained by blending or by uneven curing on the kiln, so that the criterion of satisfactory curing should not only be the average colour given by the wort alone, but a careful examination should be made for any unpleasant "back" taste detected when the first warmth of flavour passes off the palate.

It is sometimes desirable to use high diastatic material, for example, where a considerable proportion of raw grain is used, and in such cases it is almost impossible to obtain a malt with any warmth of flavour, but such material should be quite free from any sign of "rawness," as under-cured malts undoubtedly tend to give beers of low stability, and though such malts are safer to use where it is possible to counteract instability by using high mashing heats and slow fermentations, yet they are not really satisfactory. Malts of this character are therefore perhaps more often used



with safety in the stone square system of fermentation than in the ordinary skimming or dropping system.

### **Cold Water Extract, Soluble Nitrogen and Ready-formed Carbohydrates.**

There is some difference of opinion as to the precise value which the percentage of matters soluble in cold water provides in the interpretation of malt analysis, but it is generally conceded that the figure so obtained is of considerable use as a guide in determining whether a malt has been forced, or whether it has been satisfactorily treated during germination or kilning. Forced malts, by which are meant malts which, under normal mashing conditions, will produce a wort containing an amount of soluble, or more properly assimilable, nitrogen in excess of that obtained under similar conditions from an average well-made malt, certainly tend to produce thin drinking beer of lower stability than would otherwise be the case, and as such are particularly unsuitable for brewing beers that are to be kept for any length of time. It is quite conceivable that malts of this type may be used without serious danger in beers which are for quick consumption, but, inasmuch as this particular class of beer is generally one which is produced as economically as possible, other materials may also tend to instability, and it is questionable whether any real economy is served in using such a malt even in quick running mild or black beers.

The opinion that such malts are unsatisfactory was originally expressed by Moritz and Morris many years ago, and it must be admitted that recent work upon this



question, particularly the experimental work of Horace Brown on the nitrogen bodies in malt,\* strengthens this opinion. In his paper read before the Institute of Brewing in 1907,† commenting upon the question of forced malt, he says that "it is difficult to avoid the conclusion that differences observed in practice are in some way connected with an accumulation in the malt of those particular nitrogenous substances which are permanently soluble in the worts, and that there can be no doubt that the recognition of these new criteria of a quality of a malt have exercised widespread influence on malting practices during the past 10 or 12 years, and have introduced, along with more uniform and more rational methods of manufacture, a means of control which was previously unobtainable."

From a general analytical point of view it may be taken that the estimation of total matters soluble in cold water, as described, is indirectly indicative of the percentage of soluble uncoagulable proteins, and these again, as shown by Horace Brown, are in fairly constant proportion to the percentage of assimilable nitrogen compounds found in malt.

It may be desirable to discuss shortly how a forced malt is produced:—

(1) By allowing the temperature to be too high during germination, either at certain irregular periods or throughout the whole time of growth.

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\* "The Nitrogen Question in Brewing," Horace T. Brown, *Journ. Inst. Brew.*, 1909, pp. 169-296.

† "The Nitrogen Question in Brewing," Horace T. Brown, *Journ. Inst. Brew.*, 1907, pp. 394-457.

- (2) By the use of too much sprinkling water, or sprinkling too late.
- (3) By growing too short a root.
- (4) Various unsatisfactory conditions of kilning.

*Root.*—With regard to root growth, it is well known that the root is very sensitive, particularly in some seasons, and that changes of temperature quickly affect it, particularly where it is wild or straggly, and it is then easily damaged by too much turning. The growing piece should be turned only as far as is essential to prevent a rise in temperature and to allow of respiration, otherwise it should only be ploughed.

A fair amount of root is essential for the production of a satisfactory malt, and a maltster will not voluntarily make a practice of growing too much root, since this means heavy malting loss, due to excessive respiration and root growth. On the other hand, unless sufficient root is grown, the nitrogenous and other bodies which ought to be excreted by the root are left in the grain, with the result that the finished malt fulfils the conditions known as forced.

The amount of root cannot, of course, be ascertained in the ordinary analysis of a malt sample, but an amount of 12—12½ lbs. per quarter English and 10—11 lbs. per quarter for thin foreign malt barley is an average for a normally well-made malt.

*Kiln.*—Under (4) may be considered imperfect withering before loading (and that a piece should go through a genuine withering stage is of great importance), so that the piece is loaded too fresh. The grain may also be loaded in a sodden condition, which is not always due directly to over-sprinkling but sometimes to the fact that the barley is normally of low vitality and has to be loaded before it has been able to throw off its surplus moisture. Such a condition of malt, however, is more often due to excessive or too late sprinkling.

Unsatisfactory kiln construction or management, which does not allow of adequate draught, too heavy loading, or loading too deep for the amount of draught available, will also bring about a condition of stewing on the kiln and a certain increase in the matters soluble in cold water.

The temperature most suitable for drying depends entirely upon the condition of the piece and the drying facilities that are available, governed also to no inconsiderable extent by the particular atmospheric conditions; largely also upon the type of malt which it is required to produce—the more rapidly the water is driven out of the malt the lower will be the

resulting matters soluble in cold water, and the higher also will be the final diastatic power. But this must be accomplished not only with an increased temperature on the kiln, but at the same time with increased draught and, if necessary, lighter loading. Too high a temperature at the early stages of drying will produce steely, vitrified malt of most unsatisfactory flavour, and often at the same time a very high percentage of matters soluble in cold water. The temperature should not in any case be allowed to exceed  $120^{\circ}$  until the malt is hand-dry, but *given sufficient draught* the temperature may easily be brought to  $100^{\circ}$  as early as possible.

The amount of turning in the early stages of kiln drying is dependent upon the conditions of draught and temperature, for whilst much turning will facilitate the expulsion of moisture, yet this must not be done beyond the draught and heating capacity, or condensation of water will take place on the surface of the grain, with increase of the matters soluble in cold water and soluble nitrogen bodies.

As pointed out by Ling and Rendle there are factors other than that of forcing, which influence the amount of soluble carbohydrates in malt, and these authors point out that excessive respiration, in itself a condition of forced growth, may actually *reduce* their amount.

On the whole, however, and in the absence of any complete knowledge as to the actual bodies, whether of carbohydrate or nitrogenous composition, which are present in forced malts, an excessive quantity of matters soluble in cold water in a finished malt may be considered to show defective manufacture.

The standards to be adopted vary in different years—indeed, with different growths of the same year—and in the valuation of a malt it is particularly important to avoid the adoption of rigid standards.

The amount of soluble non-coagulable nitrogenous bodies should not, however, at any time exceed 2.6 per cent.

One exception to the remarks made above should be mentioned here, namely, that Horace Brown, in the paper on Nitrogen above referred to, states that no increase of assimilable nitrogen takes place during the drying stages of the malt on the kiln, but that the ready-formed sugars can rise considerably at this stage. This statement is no doubt true for malt under certain conditions, but it is difficult to believe that it always so happens, and samples of malt analysed in our laboratory taken at the withering stage just previous to loading, and also 24 hours after loading, have undoubtedly shown distinct increase in the total soluble nitrogen, which would lead one to expect that this also makes an increase in the total assimilable nitrogen.\*

## Diastase.

The diastatic power of malt is of much importance, and whilst it is impossible to give standards to cover every case, since it is obvious that the different conditions of brewing in the trade will modify the standards required, yet for general guidance the following may be adopted.

*Mild Beers.*—For this class of beer, malts must be sufficiently well cured to give a warm biscuity flavour; not only must there be warmth of flavour when the malt is first tasted, but the curing must have been so carried out as to give it a lasting flavour, though this must not have been so far as to cripple the diastase to too great an extent,

\* See also "Note on the Occurrence of High Proportions of Soluble Matters in Certain Malts," *Journ. Inst. Brew.*, 1907, pp. 132-139.

and the minimum diastatic power desirable for malts of this class is  $24^{\circ}$  (Lintner). If the diastase is lower than this the malt becomes one of high dried character and as such is only suitable for blending in a mild ale or other grist where required, but is not satisfactory for covering the malt grist as a whole, since it may result in bad mash tun conversion, and in a racking beer inclined to dryness of flavour and want of condition—this latter being a most important point in some cases, as, for example, where it is essential for finings to be completely thrown out of the bung hole (fining out). The dangers attending the use of such a malt would be still further accentuated where flaked material, raw grain or other substitutes are also used in the grist. No doubt a low mash heat assists the use of such malts, but this also increases instability and is not always practicable.

The maximum diastatic power for such a malt may be taken as  $32^{\circ}$ , and in a well modified malt thoroughly sound curing and good flavour can be obtained with such a diastase.

*Black Beers.*—For black beers or porters, much the same remarks apply as for mild beers, and though substitutes, as flaked maize, etc., are not so often included in a black beer grist, yet these are often made up of a combination of coloured malts, which require diastase for satisfactory conversion, and though as a rule lower mash heats are permissible with this class of beer, yet, where sweetness and fulness of palate are required, those malts are best not too far high dried, which are to be used in the bulk of the grist.

*Pale Ales and Bitter Beers.*—If the diastatic power exceeds  $45^{\circ}$  the malt is seldom a safe one, and either the diastase has not been properly restricted in curing, or the malt has originally been abnormally diastatic from forced growth or faulty manufacture, or it may possibly be due to the particular barleys, and barleys certainly vary in their power of producing diastase from year to year, and in some years their diastatic capacity may average high. Under normal conditions, however, this would not be the case, and pale ale malts should have a diastase of from  $32^{\circ}$  to  $42^{\circ}$  according to the particular type of pale ale required.

It should be mentioned that satisfactory conversion in the mash-tun is not alone determined by the diastatic power, but is to a large extent also governed by the tenderness of the malt, that is to say, by the completeness with which the starch cell walls have been broken down, or, in other words, modified, and a tender, well modified malt of moderate diastatic power will almost certainly permit of higher mashing heats than would a less tender malt of higher diastatic activity.

### Acidity.

The acidity of malts does not vary much; it is generally between 0.09 and 0.12, and, whilst a high acidity is certainly suspicious, many of the worst malts are low in acid.

It has already been mentioned that the acidity of malt is certainly not entirely due to lactic acid but partly to the neutral phosphates, known as diphosphates, these showing an alkaline reaction with methyl orange. According to Fernbach the presence of these phosphates retards saccharifi-



cation, whilst the acid or mono-phosphates giving an acid reaction increase diastatic activity.

Lactic and other acids when present in minute quantities in a malt or when added artificially are known to largely stimulate diastatic activity, but according to Fernbach this is due to the fact that they convert the neutral phosphates into the acid forms and so *appear* to stimulate the diastase, whereas in reality they are actually harmful. The conclusion generally arrived at is that diastatic activity is at its maximum when the solution is neutral to methyl orange.

### Saccharification.

The time taken for complete conversion of the starch should, if it were possible to make quite certain of the exact finishing stage, give valuable indication as to the completeness of modification of the malt, but it is often very difficult to make sure when testing with iodine, as described on p. 130, when all the starch present in solution has been converted into sugars. For this reason many chemists have given up this determination altogether. Some use, however, may be made of the test if it is kept strictly comparative, and as such it is a simple one, and one which can easily and quickly be carried out. In working the all-important consideration is that the malt mashed should be stirred at known definite intervals, that is, the mash should be tested 25 minutes after the original mash and stirred every 5 minutes afterwards when the wort is again tested. Under these conditions it may be of some practical use to classify malts as follows :—



(1) Extremely well modified malts, which saccharify in 25 minutes or even less time.

(2) Malts of fair average modification, saccharifying in from 25 to 35 minutes.

(3) Malts of poor modification, which do not saccharify under 45 minutes, and malts of bad saccharification, which take over 45 minutes.

Such a rough test may be of service to the brewer and give him some indication of the material he has to deal with, but the test cannot in its present form be considered suitable for including in a malt analysis.

### Sampling.

The Malt Analysis Committee, 1906, drew up the following recommendation for properly sampling malt for analysis:—

Samples sent for analysis should, so far as possible, be fairly representative of bulks, and this requires the more care when the bulks (whether from maltings or deliveries) are large, and when the malt contains any appreciable number of hard corns, and further, when there is any marked irregularity in curing.

In the case of deliveries, samples should be drawn from at least 10 sacks if the consignment amounts to over 100 sacks, or if the parcel be smaller, then from 10 per cent. of the number of sacks, but from a depth at least as far from the surface as the hand will reach when buried up to the wrist.

These bulk samples should be put into a large tin kept for the purpose, and well shaken; a smaller tin (of at least a

pint capacity) is then filled from the larger one, and sent to the analyst, the remainder being reserved in other similar small tins if the analysis is to be checked. The lids of all tins containing samples for analysis should fit well, and it is desirable as an additional safeguard in those cases in which special airtight tins are not employed to affix gummed paper round the junction of the lid and tin. Malts are really better sent in clean, carefully dried, stoppered bottles. Stoppered beer bottles answer the purpose.

In sending malts from heaps, surface samples should be avoided as in the case of sacks, and three to six samples should be withdrawn and mixed in a large tin, a small tin or bottle being filled with a portion of the mixture and sent for analysis. When the malt lies in bins a sample from the spout will generally fairly represent the bulk if the bin has been drawn upon. If not, the bin should be probed at different depths, five or six samples withdrawn, mixed in a large tin, and a small tin or bottle filled from the mixture and sent for analysis.

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## Part II.—COLOURED MALT.

The determinations to be made are extract and colour, with a physical examination.

### EXTRACT.

Methods for obtaining Laboratory Extract.—  
Of these two are available :—

(1) Mashing the coloured malt with a known percentage of an ordinary white malt containing diastase, or better with

a measured volume of cold-water extract obtained from such a malt.

(2) Extracting the coloured malt directly with boiling water.

Method (2) is inapplicable for malts which contain some proportion of unconverted starch, as for example, crystal and brown malts, but is quite suitable for black malts, or barleys in which the whole of the available endosperm contents is in a soluble condition.

The diastatic method is therefore used for determining extract in crystal and brown malts, and the boiling water method for roasted malts and roasted barleys.

The methods now to be described are based upon the report of the Malt Analysis Committee.\*

As in the case of white malt, it is desirable to use a Seck mill for grinding, which should be set with the rollers at 0.5 mm. distant from each other, but for black malts, as the Committee pointed out, the use of the Seck mill, although advisable for uniformity in crushing, might lead to error in the determination of the colour in a white malt should such be ground through the mill immediately after the coloured sample.

**Extract of Roasted Malt or Barley.**—Rather more than 50 grammes of the sample are finely ground through a coffee mill and exactly 50 grammes of the crushed malt weighed out and transferred to a 515 c.c. flask. To this 350 c.c. of boiling distilled water (or well boiled tap water will do) are added, and the malt and

\* *Journ. Inst. Brew.*, 1910, pp. 529-533.

water carefully mixed by shaking the flask in such a way as to prevent any "balling." The infusion is then kept in a boiling water bath for one hour, afterwards cooled back to 60° and made up with water to the 515 mark. It is then thoroughly well shaken and filtered through a dry paper, which must be of fairly open texture, as otherwise filtration will be found to be very slow. The specific gravity is taken in the usual way, and the extract obtained precisely as in the case of white malts.

*Example.*—The extract of a roasted malt obtained in this way gave a specific gravity of 1026·0°, equivalent, therefore, to 87·4 lbs. per quarter of 336 lbs., using the factor 3·36 as in the case of white malt.

**Extract of Brown and Crystal Malts.**—Rather more than 50 grammes are ground through a Seck mill (with the rollers 0·5 mm. apart), and exactly 50 grammes are then weighed out and transferred to a 515 c.c. flask, together with 300 c.c. of distilled water previously raised to a temperature of 158°, and 100 c.c. of cold-water extract of malt also heated before its addition to a temperature of 150°. The whole is thoroughly mixed in the flask and kept in a water bath at 150° for one hour, then cooled back to 60° and made up to the mark, well mixed, filtered, and the specific gravity taken.

The cold-water extract of malt is obtained by extracting a tender, well-modified malt having a diastase of not less than 30°, with three times its weight of distilled water for an hour at from 60° to 70°, and afterwards filtering, 100 c.c. being used as above

described for obtaining the extract of the coloured malt.

A correction must be made for the cold-water extract so added, obtained by measuring a further 100 c.c. of the malt extract into a 500 c.c. flask, adding 300 c.c. of distilled water and raising the temperature of the mixture to  $160^{\circ}$ , keeping it at this temperature for one hour, after which it is cooled back to  $60^{\circ}$ , made up to the mark, thoroughly mixed, and filtered through a dry paper. The specific gravity is then taken, and the result subtracted from that of the mixed coloured malt extract already determined.

*Example.*—A crystal malt extracted in this way gave a specific gravity of  $1028.1^{\circ}$ , and the cold-water extract  $1004.9^{\circ}$ . Then  $1028.1^{\circ} - 1004.9^{\circ} = 23.2^{\circ}$ , which is equivalent to  $77.9$  lbs. per quarter of 336 lbs.

## COLOUR.

The method adopted for determining the colour of these malts is similar to that already described for white malts, but in order to get comparative results the Committee above mentioned advise solutions of standard strength in order that there may be no variation in the depth of colour as read by different observers, for it is general experience that it is difficult to get similar results when reading solutions of very different depth of colour. The following methods are therefore adopted :—

**For Brown and Crystal Malts.**—Twenty cubic centimetres of the 10 per cent. extract solution obtained as

described, refiltered if necessary until perfectly brilliant, are measured by means of a pipette into a 100 c.c. flask and then made up to the mark with distilled water. This is thoroughly mixed and the colour taken in a 1-inch cell, using the 52 series of Lovibond's glasses.

**For Roasted Malts and Barleys.**—Twenty cubic centimetres of the 10 per cent. extract, which must be brilliant, are measured into a 1,000 c.c. or litre flask, thoroughly mixed and the colour read in a 1-inch cell, 52 series, as before.

*Examples:—*The crystal malt gave in this way a colour of 17°, so that colour in a 2 per cent. solution, 1 inch cell = 17°. The roasted malt similarly gave a colour in a 0·2 per cent. solution, 1 inch cell = 23°.

These results may, of course, be expressed if desired on the 10 per cent. solution or calculated as a percentage on the malt.

As there is some amount of red colour in many of these malts, it is found to be a distinct advantage in practice to add an 8 or 10 glass of the 52 series to the solution of malt extract, when reading its colour, and deduct this afterwards from the result found. The addition of the yellow glass in this way appears to suppress to some extent the slight red tint originally in the solution. The Malt Committee, however, advise the reading of the solution direct.

## PHYSICAL EXAMINATION.

This is most important with coloured malts. First carefully examine the corn to see whether the grain has been germinated or made up from unmalted material. This is easily



determined by an examination of the root end of the grain. Note should also be made as to the general quality of a barley, whether English or foreign, and as to whether the grain shows fair regularity of size or appears to be made up from mixed types. The sample should also be cut with a farinator, and the surface examined for general conditions of the endosperm such as evenness in roasting, crystallising, etc., and the percentage of burnt or charred corns. Careful note must be made of the aroma and flavour, which is best obtained by grinding a little of the malt in an ordinary hand mill and examining the crushed material. Both aroma and flavour are of very great importance in coloured malt analysis.

## INTERPRETATION OF THE ANALYSIS OF COLOURED MALTS.

**Black Malt and Black Barley.**—There is some difference of opinion amongst brewers as to the relative advantages of roasted malt and roasted barley, and whilst it is true that a roasted barley is the more economical purchase, yet it is generally agreed that this latter tends to give a somewhat coarser and dryer flavour to the beer than does a roasted malt, which, on the contrary, will produce a fuller, softer, and sweeter palate flavour.\*

It is the opinion of some brewers, moreover, that the colour of a beer as obtained from a grist containing roasted barley is not so permanent as that resulting from one containing roasted malt.

\* Reference should be made to a paper on Coloured Malt by Lawrence Briant, *Journ. Inst. Brew.*, 1907, pp. 486-501.



The choice, therefore, is determined not only by cost of materials, but also by considering the particular class of black beer which it is required to produce, but on the whole for running black beers or porters, roasted barley should prove quite as useful as roasted malt, particularly as these beers are generally consumed only when they have been primed with sufficient sweetness to cover any roughness or coarseness of flavour.

It has hitherto been the custom for coloured malts to be sold by volume instead of by weight, and, as a result of this confusing system, the actual weights of equal volumes of different coloured malts are found to vary considerably. The system has the disadvantage of enabling the maltster to roast the barley in such a way as to increase the volume measure without improving its brewing value, and it certainly increases the difficulty of obtaining comparative valuations for different malts. It has been suggested that it would be very much more convenient if all coloured malts were sold on a given weight basis, frequently taken as 280 lbs. to the quarter, for only in this way can satisfactory comparative values be obtained between the laboratory determination of extract, colour, etc., and practical brewing results.

*Moisture.*—The percentage of moisture in coloured malt should not be permitted to exceed 4, and it must be remembered that this class of malt very quickly absorbs moisture, and, when actually slack, is very liable to cause trouble in brewing. For these reasons, brewers generally arrange to keep only a small stock of this material and get it delivered to the brewery in much

smaller consignments than is the case with ordinary white malt; at the same time the aroma is best obtained from perfectly fresh malt, as when such malt becomes slack it very quickly loses its aroma value.

*Extract and Colour.*—The analytical examples given show that black malts and barleys may vary considerably, both in extract and colour. Black malts in all cases give higher extracts than black barleys, as might be expected, and it may be taken as a general rule that the more tender and more completely modified the barley, the higher will be the resulting extract, though this also, to some extent, depends upon the care and completeness with which the malt or barley has been roasted. The surface of the grain should show the endosperm evenly and uniformly roasted, and the amount of charred or carbonised corns, which are easily distinguished, should be small, as such corns mean loss of extract and produce a bitter flavour. The ground malt should be pleasantly aromatic, and the flavour, which is best obtained by tasting the *ground* sample, should be as free as possible from any marked bitterness. As this class of material is only used in comparatively small proportions in the brewing grist, usually not more than 5 per cent. and rarely exceeding 10 per cent., the question of the amount of extract is evidently of less importance than that of flavour and aroma. The colour of these malts will vary very considerably in their particular tint of red and black, and many brewers are very particular as to the exact shade of colour such a malt will give, this being a very important matter, where it may happen that particular stress is laid upon the uniformity of colour of a black beer. In this

respect the brewer should be warned against the error of judging the colour of such material by the appearance only of the ground sample. Results obtained comparatively in this way are often very misleading, and the only satisfactory method is to actually obtain the extract of a malt as described, and from it determine the colour by some standard method, of which the Lovibond Tintometer is by far the most reliable one.

**Crystal Malts** should be tender, and the cut surface of the corns should show an even and complete crystallisation. Flavour and aroma are, of course, most important, and the best class of crystal malt is that which has a peculiarly luscious sweetness on the palate. Some crystal malts contain a proportion of corns which have been over-crystallised, and these will give a dryer flavour and are more similar in character to the ordinary high-dried malt.

Crystal malt is used in the grist, both for mild ales and also for black beers. The amount used in the former varies from 5 to 15 per cent. of the total malt grist, and in some cases even still higher percentages are used. Whilst there is no doubt that, when used in fairly large percentages, a good crystal malt will give a character and quality to the beer not otherwise obtainable, these crystal malts vary considerably, and it is better to use a small percentage of a really high class and well-made crystal malt than a larger percentage of one of inferior quality.

When used in black beers the percentage averages from 5 to 10.

Brown or amber malts are sometimes used in a black

beer grist for obtaining quality and character of flavour, as is also torrefied barley, but this latter is more often added to ale grists.

The following analyses are a few examples of some of these malts, the figures of which were obtained by the methods described :—

|                    | Extract<br>per 336 lbs. | Colour<br>2 per cent.<br>Solution. | Colour<br>0·2 per cent.<br>Solution. |
|--------------------|-------------------------|------------------------------------|--------------------------------------|
|                    | lbs.                    | °                                  | °                                    |
| Crystal malt ...   | 83·6                    | 31                                 | —                                    |
| „ ...              | 82·4                    | 21                                 | —                                    |
| „ ...              | 79·3                    | 33                                 | —                                    |
| Brown malt ...     | 78·6                    | 40                                 | —                                    |
| Roasted malt ...   | 87·4                    | —                                  | 23                                   |
| „ ...              | 83·1                    | —                                  | 28                                   |
| Roasted barley ... | 80·6                    | —                                  | 30                                   |
| „ ...              | 69·5                    | —                                  | 24                                   |

### Part III.—BARLEY.\*

Not much is to be gained by making a chemical determination of all the constituents of barley, but it is

\* Barley should of course be more properly considered in the next chapter, but it is conveniently included here in its relation to malted grain.

For studying the anatomy, general botany and classification of barleys, the following papers should be consulted :—“Researches on the Germination of Some of the Gramineæ,” H. Brown and Morris (*Journ. Chem. Soc.*, 1890, p. 466); “Varieties of Barley,” E. S. Beavan (*ibid.*, 1902, pp. 542-594); “Laboratory Studies for Brewing Students,” Adrian Brown.

often desirable to ascertain the moisture percentage, the germinative power, and sometimes also the nitrogen. Some notes are therefore given referring to these determinations, which are made as follows :—

### MOISTURE.

The moisture is determined exactly as in the case of malt ; 5 grammes of ground barley are dried for five hours in the water bath, cooled in the desiccator and weighed, the loss being calculated as moisture.

This determination of moisture is of considerable importance, and the percentage found in barleys will depend upon the general and local conditions of harvesting in any particular season, and also to some extent upon the degree of ripeness of the grain. Where harvest conditions have been bad a high percentage of moisture may be found, together with probably a considerable degree of unsoundness—this apart from the question as to whether the barley has been originally well ripened or not, and if there is at the same time unripeness, the conditions of unsoundness will be intensified.

No doubt satisfactory ripening is dependent to a very large extent upon the degree of desiccation or drying, and the optimum ripening is obtained when there is a minimum of moisture. A. D. Hall\* points out that, in the case of wheat, ripening takes place up to a much later stage than is generally supposed—within a week or so of harvesting.

It was at one time thought that an unripe barley could easily be recognised by the greenish appearance of the

\* *Journ. Roy. Agric. Soc.*, 1909.

corns, but Adrian Brown has shown that the green colour is naturally characteristic of certain types of barley, and is sometimes due to the colour of the aleurone cells as these are seen through the outer coat or testa of the barley grain. Unripe barley is obviously unsatisfactory for malting, and apart from its unsoundness will certainly produce a malt of relatively low extract.

Over-ripeness is also objectionable, and such barley does not produce the best malt; it is usually very pale and colourless in appearance.

In a good season English barleys may average 10-12 per cent. of moisture, but in some years they may contain as much as 18 or even 20 per cent.

**Kiln Drying.**—Under these conditions kiln drying is essential or the grain will quickly heat and be unfit for malting. The usual temperature for kiln drying is about 100 to 110° F., and the grain is loaded on the kiln up to about 6 inches in depth, and is dried for about 24 hours.

Kiln drying should reduce the moisture percentage of a barley to 12 per cent., and if the barley is to be kept over for any length of time, it is better reduced to 10 per cent. Even where a barley contains not more than about 12-14 per cent. moisture, kiln drying is generally considered advisable, for it must be remembered that the percentage of moisture is only an average one, and is unlikely to be distributed evenly throughout the whole of the grain, and further, there is no doubt that the equalising of moisture by kiln drying will produce greater regularity in the growth after steeping.

A further consideration is in the fact that kiln drying



undoubtedly produces what is known as maturation or an artificial ripening, and it was proved by Beavan that a percentage of the water lost in kiln drying was not accounted for, this having combined, probably in some way chemically, with the constituents of the barley grain, and so resulted in a definite chemical change.

It is important to remember that kiln-dried barleys must be kept for at least three or four weeks before steeping, as otherwise they are better wetted without undergoing the process.

It has been mentioned that desiccation is probably an important factor in the ripening of barley as it is in wheat, but there is no doubt also that transformation of non-protein matter into protein also takes place, and the optimum ripening of a barley may be considered to have been obtained when such barley contains a minimum of moisture and a maximum transformation of sugars to starch and insoluble proteins into soluble forms.

## GERMINATIVE POWER.

The barley to be examined is steeped in the usual way, and 100 or more corns are grown in some form of germinator. Not only must observation be made as to the number of corns which finally grow, known as the "*germinative capacity*," but note must be taken as to the regularity with which the corns chit or show rootlet, and the time taken—" *germinative energy*."

It is sometimes more useful to grow a number of steeped corns in moistened flannel and to examine these from



time to time. This method allows of a considerable bulk of barley being taken, and serves as a useful alternative to the above.

The rate at which the barley will germinate has been considerably discussed in recent years in relation to the question of aëration in the steep. This matter has been thoroughly dealt with in a paper by Baker and Dick,\* to which reference should be made, and there is no question that aëration of the steep, if this is *thoroughly* carried out by actually blowing in compressed air or otherwise completely aërating the grain, will produce an earlier chitting on the floor, so that a day may often be saved in the flooring period. How the aëration affects the slower growing proportion of the grain is not so apparent, nor whether this helps ultimately to improve the germinative capacity.

The total loss on the dry barley due to germination is found to average 11·0 per cent. or rather less, of which approximately 4·2 per cent. is loss due to rootlet growth, the root containing about 5 per cent. of excretory nitrogen.

## NITROGEN.

About 5 grammes of the barley are finely ground in a small mill, and 1 gramme accurately weighed out and transferred to an 8-oz. flask. Ten grammes of potassium sulphate and 20 c.c. strong sulphuric acid are added, and the process continued exactly as in the determination of nitrogen in malt (p. 119).

A high nitrogen barley is said to produce a malt con-

\* *Journ. Inst. Brew.*, 1905.

taining a high percentage of soluble non-coagulable proteins, but this latter figure is determined so largely by the conditions of malting that the point is a difficult one to prove.

The total nitrogen in barley will vary from 8 to 14 per cent., expressed as protein on the dry barley.

It is generally considered that malted foreign barleys, as a whole, tend to give rather thinner-drinking beers than do English, but the writer is not sure that this may not be due, partly, at any rate, to the readiness with which much foreign barley will grow, and the danger of over-modifying it. Some foreign barleys contain quite as high a total nitrogen percentage as English, but under equal conditions of malting an English malt will certainly contain a higher percentage of soluble non-coagulable nitrogenous bodies than will a foreign.

For this reason, and because foreign barleys are grown under so much more favourable conditions for uniform ripening, foreign malts are of value where increased stability, less tendency to throw deposit, and such properties are required.

The *total* protein value of barley must remain of little practical value until the separate nitrogenous constituents of which the total protein matter is built up have been isolated and their properties determined.\*

\* See some recent researches by H. T. Brown and others, *Trans. Guinness Research Lab.*, 1903.

## CHAPTER IV.

**UNMALTED GRAIN.**

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**RAW GRAIN.**

THIS usually consists of maize or rice grits, and analysis is chiefly useful for showing the amount of brewing extract obtainable when an infusion of such material is made with malt, or extract of malt, containing diastase.

The determination of the starch percentage is an indication of the extract value of the material, but for practical purposes the estimation of the starch products due to diastatic action upon the starch present in the grain, by a method closely comparable to the process usually adopted in the brewery, is much more simple and gives results which from the brewer's point of view are really more accurate. A method for obtaining the "brewing extract" is therefore described, also one for the determination of oil in the sample, which is important, and the moisture, mineral matter and, in some cases, the soluble nitrogenous bodies may also be estimated.

Two methods are also given for the determination of starch, but all these at present known are both laborious and difficult.

The soluble nitrogenous matters are quite insignificant in amount, except in damaged and heated grain, when occasionally some portions of the gluten may have been

changed and rendered soluble. The determination of this figure is useful, therefore, when examining suspected samples.

**Brewing Extract.**—Twenty grammes of the finely ground sample are carefully weighed out and transferred to a conical-shaped flask of about 250 c.c. capacity. About 110 c.c. of cold distilled water are added, together with 5 c.c. of cold-water extract of malt (approximately 33 per cent., and obtained as described on p. 149).

The flask and contents are then slowly heated in a water bath up to boiling point, and during the rise of temperature it is essential to keep the contents of the flask continually stirred, as otherwise the grits may cake together and refuse afterwards to completely gelatinise, with subsequent loss of extract (the stirring may be effected by means of a glass rod dipping into the flask through a small funnel with the stem broken off).

After boiling for one hour, the contents of the flask are cooled back to  $160^{\circ}$ , and a further 45 c.c. of the cold water extract of malt solution then added, thus completing the total amount of 50 c.c. The whole is allowed to stand for two hours at  $150^{\circ}$ , after which it is cooled back to  $60^{\circ}$ , washed into a 200 c.c. flask, made up to the mark, thoroughly shaken, filtered, and the specific gravity taken in the usual way.

A correction must now be made for the cold water extract of malt added, and this is obtained by measuring 50 c.c. into a 200 c.c. flask, adding about 100 c.c. of water and digesting this alongside the grain mash for two hours at  $150^{\circ}$ .

The solution is then cooled back to  $60^{\circ}$ , made up to the 200 c.c. mark with water, thoroughly mixed, filtered, and the gravity taken. This is subtracted from that already obtained for the mixed grain and malt extracts.

An example will make this clear.

A sample of maize grits, mashed as above described, gave the following results :—

The grits and malt extract had a specific gravity of  $1036\cdot25^{\circ}$ , obtained from an average of two determinations. The extract of malt alone, 50 c.c. in 200, had a specific gravity of  $1004\cdot85^{\circ}$ .

Then  $1035\cdot25 - 1004\cdot85 = 30\cdot40$ .

It will be remembered that with malt extract we used the factor 3·36 to obtain the extract per quarter, but in this case the factor 3·32 is used, obtained from the factor 3·36 by multiplying this latter by  $200/202\cdot5$ , since it is necessary to allow  $2\frac{1}{2}$  c.c. for the volume occupied by the cellulose and other substances of the grain.

Therefore  $30\cdot4 \times 3\cdot32 = 100\cdot9$  *lbs.* extract *per* 336 *lbs.* of the grits.

**Oil.**—This is best estimated in a Soxhlet fat extraction apparatus,\* as follows :—

\* Dr. Skalweit's form of 60-gramme size is suitable. The complete fat extraction apparatus consists of (A) the Soxhlet, (B) a flask attached to its base, and (C) a condenser fitted to the top. The ether or other volatile solvent is heated in the flask, the vapour rising through the side tube of the Soxhlet to the condenser, where it is condensed, and, falling on to the sample, extracts the oil. The condensed ether gradually fills the extractor, rises to the level of the upper limb of the syphon, when it is returned back to the flask. The process is continuous. By allowing the sample to soak in the ether over-night, the



SOXHLET FAT EXTRACTION APPARATUS.





Insert in the bottom of the tube of the Soxhlet apparatus a loose plug of glass wool or fat-free cotton wool. Weigh out 15 grammes of the finely ground grain and brush into a thimble-shaped receptacle, which may be made by shaping filter paper round a test-tube of wide diameter, or may be purchased, but in the latter case the filtration is apt to be rather slow.

Cover the grain with sulphuric ether, and attach to condenser. Now place about 50 c.c. of sulphuric ether in the receiver flask, and connect with the apparatus. Allow to stand over-night. The next morning immerse the flask in a bath of water, heat gently by means of a Bunsen, and distil the ether through the sample for four hours, when all the fat will have been extracted.

Disconnect the flask, and, if necessary, filter the ethereal liquid through a small filter paper, washing both the flask and the filter with a few drops of ether. Collect filtrate in a tared beaker, or wide-mouthed flask, and cautiously evaporate to dryness over water bath. Place in oven of bath, and weigh after half an hour. Replace in oven, and again dry and weigh. Repeat this till constant.

Calculate to percentage of material.

*Example :—*

Fifteen grammes taken. The oil after extraction and evaporation weighed :—

extraction the following morning is shortened. Great care must be taken that there is no leakage in any of the connections of the apparatus, as ether is extremely inflammable and would immediately take fire should any of its vapour come in contact with a flame.

---

|                |     |     |                 |
|----------------|-----|-----|-----------------|
| Beaker and oil | ... | ... | 15.841 grammes. |
| Tare of beaker | ... | ... | 15.530 grammes. |
|                |     |     | <hr/>           |
|                |     |     | 0.311 gramme.   |

Then  $0.311 \times \frac{100}{15} = 2.07$  per cent of oil.

**Starch.**—A number of methods have been suggested at various times for the estimation of starch in cereals and other food substances, but none of these appear to combine accuracy with quickness of working, whilst it is usually found that a process which works quite satisfactorily for certain classes of food material is not applicable to others.

The methods here described have both given good results for the determination of starch in raw and prepared grain. The first method, that of Ewer, is much more rapid and more easily performed than is the second method, O'Sullivan's.

**\*Ewer's Method.**—For maize or rice the procedure is as follows :—Twenty-five c.c. of glacial acetic acid are run into a 200 c.c. flask without wetting the neck. Five grammes of the finely ground material are then added, and the flask closed and vigorously shaken until the mixture is uniform. The stopper and neck of the flask are then washed down with a further 20 c.c. of glacial acetic acid. The flask is next placed in a boiling water bath for 10 minutes, then 10 c.c. of dilute hydrochloric acid, 1 in 10, are added, and the flask left in the water bath for exactly six minutes, being shaken round every minute. Hot water is then added to make the volume up to 180 c.c., and the mixture is heated for a further 15 minutes in the boiling water bath. The solution is finally cooled, clarified by the addition of 2.5 c.c. of potassium ferrocyanide, made up to the mark with water,

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\* For both of these methods the student must be thoroughly conversant with the use of the Polarimeter, see p. 400.

filtered and polarised. If the filtrate cannot be obtained clear, a few crystals of zinc sulphate can be added to assist clarification.

Ewer found that corrections were necessary owing to the presence of optically active bodies other than starch. A blank experiment is therefore necessary, and is conducted as follows :—

Five grammes of the finely ground material are added to 100 c.c. flask with 70 c.c. of water at about 120°, and the whole thoroughly mixed by vigorous shaking. This is digested at the ordinary temperature for one hour, then 25 c.c. of glacial acetic acid are added, and the digestion continued at the same temperature for half an hour, during which the temperature is adjusted to 60°, the ferrocyanide added as before, and the liquid made up to the 100 c.c. mark, mixed, filtered and polarised.

The specific rotatory power  $[\alpha]_D$  at 68° C. is, for rice, 186·07, and for maize 184·19, which values are obtained on the 5 per cent. solution, but they are practically constant for different concentrations.

*Example :—*

After making the necessary correction for the angle obtained in the blank experiment it was found that the solution gave an angle of +132·3° per cent. of the material.

Therefore the percentage of starch =  $132\cdot3 \times 100 \div 184\cdot2$ . The maize grits has therefore a starch percentage of 71·8.

**C. O'Sullivan's Method.**—This depends upon the gelatinisation of the starch, and its subsequent conversion by the diastase of cold malt extract into maltose and dextrin. It is first necessary to remove the oil and the small amount of soluble carbohydrates present.

For this purpose, take an average sample of the grain, grind about 50 grammes to a powder in a mill, and well mix. Carefully weigh out 5 grammes, and place it in a small dry bottle or flask with about 20 to 25 c.c. of sulphuric ether, cork the flask, and occasionally shake during about four hours. At the end of this time pour off the ether through a small dry filter paper, and wash once or twice more with ether. This will remove the oil from the sample. The oil may also be extracted in the Soxhlet apparatus, as described further on; either method answers equally well. Now place the dis-oiled grain in a beaker—washing in any that may be on the paper with a little drop of water—pour on about 1 litre of cold water, and stir well. Allow to stand about 24 hours, then decant the clear, supernatant liquid through a filter, and wash once or twice with lukewarm water (about

95° F.). The grain is now carefully transferred to a small beaker (about 100 c.c.), using for this purpose not more than 40 c.c. of water. Surround the beaker with warm water, which gradually heat to boiling, well stirring the mixture of grain and water to prevent any lumps being formed. Keep in the boiling water for about ten minutes, then cool to 145° F., and add 10 c.c. of a strong extract of malt obtained by just covering 100 grammes of finely ground pale malt with cold water, standing for a few hours and filtering bright.

It will be necessary to ascertain and deduct the corrections due to any copper-reducing bodies, etc., present in the malt solution, so that a further 10 c.c. of this with about 40 c.c. of water are digested alongside the starch experiments and the estimations conducted in the same way, the extract finally being made up to 200 c.c. and the reducing sugars and optical activity determined for making the necessary correction.

Keep the mixture at 145° to 150° F. for some time (about one hour), until all the starch has become saccharified, and no trace of starch is shown on testing a drop or two with iodine; then raise to boiling, and boil for 10 minutes. Cool, and make up to 200 c.c., and filter through a dry paper into a dry beaker. The solution now contains the conversion products from the starch in 5 grammes of the grain, and the precise amount of these may be ascertained from the cupric oxide reducing power and the optical activity. The determinations are carried out as follows:—

**Copper Reducing Power.**—This estimation is made exactly as described in "Sugar Analysis," 10 c.c. of the solution being added to the Fehling mixture (15 c.c. copper sulphate solution, 15 c.c. alkaline tartrate solution, 50 c.c. water), and the determination carried through as given in ascertaining the invert sugar by the gravimetric process.\*

**Optical Activity.**—The angle may be taken direct from the solution.

In order to calculate the starch from the figures thus obtained we proceed as follows:—

First make a correction for the weight of CuO found by subtracting the weight of CuO due to the malt extract, and multiply the CuO thus obtained by 0.7435 to obtain the maltose; this result multiplied by 20 will give maltose in the 200 c.c., or 5 grammes of grain, which again multiplied by 20 gives the percentage of maltose.

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\* See p. 194.

Now multiply the angle by 40 (having made the necessary correction by subtracting the angle due to the cold-water extract from the angle obtained for the starch conversion solution) and thus find the angle of the products. Now, as all starch hydration products resulting from the action of diastase may be expressed in terms of maltose and dextrin, it is only necessary to calculate the angle due to maltose, deduct this from the total angle, and calculate the residual angle to its equivalent of dextrin.

Each 100 parts of maltose are derived from 95 parts of starch,\* but there is no gain in weight in the production of dextrin. We merely, therefore, multiply the maltose by 0.95, and add the figure so obtained to the dextrin, the result being the amount of starch.

*Example :—*

Copper reducing power—10 c.c. solution used :—

CuO obtained = 0.198.

Then  $0.198 \times 0.7435 \times 20 \times 20 = 58.88$  maltose.

Optical activity :—

Reading in 100 mm. tube =  $3.2^\circ$ .

Then  $3.2 \times 40 = 128^\circ$  total angle.

The angle due to maltose equals  $80.0^\circ$  ( $58.88 \times 1.359$ ), and this deducted from the total angle equals—

$128 - 80 = 48^\circ$  due to dextrin,

and  $48 \div 1.944 = 24.69$  dextrin.

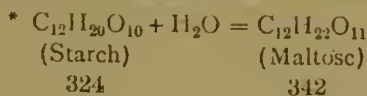
The starch is calculated from these figures as follows :—

Maltose  $58.88 \times 0.95 = 55.93$  starch.

Dextrin 24.69 = 24.69 starch.

80.62

giving starch in grain 80.62 per cent.†



so that 342 parts of maltose are produced from 324 parts of starch, or 100 parts of maltose from 95 parts of starch.

† In this example the corrections due to the cold-water extract of malt are not given.

**Mineral Matter.**—Take 5 grammes of the substance in a tared platinum dish, placed on platinum wire or pipe-clay tripod over Bunsen burner, and apply heat cautiously, at first, and when only a charred mass is left, ignite more strongly, covering the dish with a piece of platinum foil. Burn off until the ash is grey, or white, and weigh.

*Example :—*

5 grammes of maize grits were taken.

|              |     |     |                 |
|--------------|-----|-----|-----------------|
| Dish and ash | ... | ... | 32.517 grammes. |
|--------------|-----|-----|-----------------|

|            |     |     |                 |
|------------|-----|-----|-----------------|
| Dish alone | ... | ... | 32.502 grammes. |
|------------|-----|-----|-----------------|

---

0.015 gramme.

and  $0.015 \times 20 = 0.30$  per cent. of mineral matter.

**Nitrogenous Matter.**—One gramme of the grain is taken, and the determination conducted as described in the “Analysis of Malt,” p. 119.

Should it be desired to estimate the soluble nitrogenous bodies, this may be done by taking 100 grammes of the grain, digesting it for four hours with 400 c.c. of cold water, frequently stirring the mixture during the stand. At the end of the time, filter the liquid bright through a dry paper, into a dry beaker. 100 c.c. is now taken, placed in a flask, and evaporated, the determination then being continued as before. The results are calculated to percentage on the grain.

**Moisture.**—Five grammes of the finely ground sample are weighed out in a tared dish, dried in the boiling water bath for five hours, cooled in the desiccator and again



weighed. The loss in weight multiplied by 20 gives the percentage of moisture in the sample.

*Example :—*

|                                |     |     |                   |
|--------------------------------|-----|-----|-------------------|
| Dish + grits ...               | ... | ... | = 33·709 grammes. |
| Dish + grits after heating ... | ... |     | = 33·099 grammes. |
|                                |     |     | <hr/>             |
|                                |     |     | 0·610 gramme.     |

and 0·610 multiplied by 20 = 12·2 per cent of moisture.

## PREPARED GRAIN.

These brewing adjuncts (gelatinised or “flaked” rice, maize, or barley) are starch-containing substances, which have been subjected to treatment to gelatinise the starch, and render it readily saccharifiable by diastase at ordinary mashing temperatures. When used they are therefore mixed with the grist, and mashed without any preparatory treatment in the ordinary manner. In the analysis of these materials the following determinations are made :—

1. Brewing extract.
2. Oil.
3. Moisture.

In addition, the nitrogen, starch, cellulose, and mineral matter may, if desired, be also determined.

**1. Brewing Extract.**—The theoretical yield of extract may be calculated from the amount of starch contained in the material; but this amount is seldom realised in practice, for in many samples of prepared grain there is



some ungelatinised starch, and occasionally some also which, though gelatinised, has been converted in the subsequent drying process into a vitrified and horny condition, and is then not readily saccharified by diastase.

The extract is determined\* by a process similar to that just described for raw grain, but as the starch in the case of prepared grain should be in a gelatinised condition, it is unnecessary to bring the grain to the boiling temperature as was done with the maize or rice grits. The determination, which should always be done in duplicate, is made by weighing 20 grammes of the flaked material and transferring to a beaker of about 250 c.c. capacity, adding 120 c.c. of distilled water and raising the temperature of the whole in a water bath to 160°. Fifty cubic centimetres of the cold-water extract of malt, the preparation of which has already been described, are then run into the beaker, keeping the whole well stirred with a glass rod (which is afterwards rinsed down with distilled water), and the beaker covered with a clock glass and kept in a water bath at a temperature of 150° for two hours. At the end of this time it is washed through a small funnel, which should have a large orifice, into a 200 c.c. flask, cooled back to 60° F., made up with distilled water, thoroughly mixed, and filtered through a dry filter. The

\* In the last edition of this book the extract was determined somewhat differently, the grain being mashed with 75 per cent. of a tender malt, but it was found that under these conditions the extract obtained from the grain varied with the particular malt used, depending upon the diastase and tenderness of the sample. Variations are less frequent when using a malt extract, but care must still be taken that the malt chosen is fairly diastatic and at the same time well modified and tender.

specific gravity is then taken, and a correction made for the malt extract as described, exactly as in the case of the unprepared grain.

*Example:—*

A sample of “flaked maize,” mashed as above, gave the following results:—

Maize extract, together with malt extract, gave a specific gravity of  $1037.25^{\circ}$

Malt extract alone  $1005.50^{\circ}$

---

 $31.75^{\circ}$ 


---

and this multiplied by the factor  $3.32 = 105.4$  lbs. extract per 336 lbs. of flaked maize.

2. Oil

3. Moisture

} Are determined as in raw grain, as are

also the starch, nitrogen, and mineral matter, but these are seldom required.

**Cellulose.**—It is rarely necessary to directly estimate cellulose in either raw or prepared grain, but where this is required the following method is found to answer satisfactorily:—

10 grammes of the substance are finely ground and treated in a small flask, with 50 c.c. of 5 per cent. sulphuric acid, and 150 c.c. of distilled water. Boil for half an hour under a reflux condenser, allow to settle, and pour off the bright liquor. Boil the residue again twice with 150 c.c. of water, pouring off as before.

To the residue so obtained is now added 50 c.c. of soda solution and 150 c.c. of water. This is then treated exactly as in the case of the acid, and the residue obtained from the soda extraction is finally washed on to a weighed filter paper, where it is thoroughly washed with water, alcohol, and ether. It is then dried in the water bath until the weight is fairly constant. The filter paper and contents are finally burnt off,

and the weight of ash so obtained deducted from the total weight. The result, multiplied by 10, gives the cellulose or crude fibre in the cereal.

*Flaked and malted oats* may be analysed in a similar manner to flaked maize or rice. There is usually some amount of diastatic activity in malted oats, and where this is sufficiently high the extract may be obtained as in the case of white malt, but the diastase is often very low, and it is safer therefore to determine the extract as described for flaked grain.

## INTERPRETATION OF ANALYSES OF RAW AND PREPARED GRAIN.

**Raw Grain.**—Raw grain, as used by brewers, is usually either maize or rice, though occasionally barley is also employed. Maize is generally supplied in the form of grits. In this preparation the husk and germ have been more or less completely removed, and, in consequence, the proportion of the oil, which is situated largely in the germ, is diminished, as is shown by the following figures of Fawcett\* :—

|   | Oil per cent. |
|---|---------------|
| Germ, and surrounding oily portion ...                                    | 21·0          |
| Centre, or starchy portion, closely surrounding the germ or oily part ... | 3·4           |
| Main portion of the grain, constituting the flinty or horny portion ...   | 0·8           |
| White portion, situated farthest from the oily part ...                   | 1·4           |
| The maize sample ground ...   | 4·8           |

\* *Journ. Inst. Brew.*, vol. 2, p. 378.

The oil of maize is certainly objectionable from a brewing point of view, and although in some converting, or, more strictly, gelatinising, apparatus, the oil may be driven off, yet in most cases the oil present in the material is not afterwards removed. Maize grits should not, therefore, contain more than 1 per cent. of oil. In consequence of the degermination the proportion of proteins is somewhat decreased, whilst the percentage of starch is raised. The following figures should be given:—

|          |     | Whole Maize. |     | Maize Grits. |
|----------|-----|--------------|-----|--------------|
| Starch   | ... | 62 per cent. | ... | 74 per cent. |
| Proteins | ... | 9 „          | ... | 8 „          |

There appears to be a preference for European over American maize, but in the best samples of the latter there is certainly no inferiority as measured by analysis. British manufacturers of flaked maize insist, however, that their best produce is always obtained when using grain of European growth, and the opinion of practical manufacturers on this point must certainly have weight, particularly when the fact is remembered that European is dearer than Transatlantic grain.

The theoretical extract obtainable from maize grits lies between 96 and 100 saccharometer lbs. per 336 lbs. The practical extract may be taken as a little less. Statements as to the possibility of obtaining in practice an extract of 105 to 110 lbs. per 336 lbs. may be regarded with some distrust.

Rice is a more starchy material, and, therefore, capable of yielding considerably higher extract. As delivered into

this country it is already dehusked, and is used as raw grain with no treatment other than grinding. It should contain about 79 per cent. of starch, not more than 0.4 per cent. of oil, or 7.5 per cent. of proteins. It is capable of giving a laboratory extract of 103 lbs. per 336 lbs. Owing to the fact that its starch gelatinises with considerably more ease than that of maize, it is easier to obtain the full extract. The amount of moisture in maize grits and rice should not exceed 14 per cent.\*

Barley is becoming increasingly used as raw grain. On account of its husky character it is less liable to give trouble with drainage in the mash-tun than maize or rice, and although it, of course, gives a lower extract, yet half-corn and samples which contain too many dead corns for malting are frequently used, and constitute a cheap form of extract. The extract obtained from barley should be about 88 lbs. per 336 lbs., the proportion of oil should not exceed 2.5 per cent., and the amount of nitrogenous matter 12.5 per cent. If kiln-dried it gives a flavour not obtainable from other raw grain.

In the case of both raw and prepared grain, it must be admitted that there is necessarily some loss of malty flavour in the beers produced with them. This loss is, however, in practice almost inappreciable if only small proportions are used, the only difference in flavour perceptible being an increased cleanness of palate, whilst more rapid brightening and conditioning are very generally experienced. If large proportions are employed, some yeast weakness, particularly sloppiness, will often assert itself, presumably owing to the

\* Grits should be quite free from any rancid smell or flavour.

reduction of nitrogenous matter in the wort, for, though the grain contains a considerable amount of nitrogenous matter, it is practically all insoluble, and is not rendered soluble, or, at any rate, not to any appreciable extent, under the influence of the enzymes acting during mash-tun operations.

With flaked materials (sometimes incorrectly termed "flaked malt") not only is the excess of oil and nitrogenous matter removed, but the starch granules are, or should be, in a gelatinised condition. The flaking operation is carried out as follows :—

The maize, rice, or barley, after separation, if necessary, of excess of oil, is soaked in cold water for several hours until the starch cells have absorbed water and swollen up. It is then conveyed through a steaming apparatus, travelling slowly, by means of a screw, along a cylinder, into which steam is blown. The starch cells are thus burst. The grain is now crushed between hollow steel rolls, heated by means of steam, and is thus at once flaked and dried. This drying requires, however, to be supplemented by subsequent treatment on the kiln, or by means of a hot-air blast blown through the nearly dried material. The flakes should be thin, and the starch cells should all of them have been ruptured. The proportion of water will be reduced to about 4 per cent., but the dry flakes very rapidly absorb moisture, so that as delivered into the brewery they seldom contain less than 6 per cent.—often more. The following standards may be adopted for flaked materials :—



|                           | Maize.         | Rice.          |
|---------------------------|----------------|----------------|
| Starch, not less than ... | 75·0 per cent. | 80·0 per cent. |
| Fat, not more than ...    | 1·3 „          | 0·5 „          |
| Proteins, not more        |                |                |
| than ...     ...     ...  | 10·0 „         | 8·5 „          |
| Moisture, not more        |                |                |
| than ...     ...     ...  | 8·0 „          | 8·0 „          |

It is not satisfactory to calculate extract from starch proportion, for often some of the starch granules may not have been properly gelatinised, or, if gelatinised, may, by improper treatment, have become in a horny condition, exceedingly refractory in conversion, so that it is seldom that in practice the extract will be obtained equal to that calculated from the starch. In connection with the use of raw and flaked grain an important advantage, quite apart from any financial one, must not be overlooked. Where pale beers are required, the brewer is often tempted to employ malts so pale in colour that there is much danger of actual undercuring. By the use of these substitutes, however, a much higher dried, better flavoured, and more satisfactorily cured malt may be used, for the extract from raw or flaked grain, even with the yellow maize, is practically colourless. Thus it may happen that, when using a small proportion of substitute, a beer of actually more malty flavour may be obtained, the added curing of the malt more than compensating for the loss of flavour in the unmalted grain.

*Oats.*—Flaked oats give an average extract of 88·5 lbs. per quarter, as does oatmeal. Malted oats from 70 to 76 lbs. per quarter.



Oats when added to a grist in appreciable percentages distinctly increase the richness of flavour, and are particularly valuable for black beers.

### Composition of Cereals.

The following are from analyses by Graham :—

|                      | Old<br>Wheat. | Barley. | Oats. | Rye.  | Maize. | Rice. |
|----------------------|---------------|---------|-------|-------|--------|-------|
| Water ...            | 11.1          | 12.0    | 14.2  | 14.3  | 11.5   | 10.8  |
| Starch ...           | 62.3          | 52.7    | 56.1  | 54.9  | 54.8   | 78.8  |
| Fat ...              | 1.2           | 2.6     | 4.6   | 2.0   | 4.7    | 0.1   |
| Cellulose ...        | 8.3           | 11.5    | 1.0   | 6.4   | 14.9   | 0.2   |
| Gum and<br>Sugar ... | 3.8           | 4.2     | 5.7   | 11.3  | 2.9    | 1.6   |
| Proteins ...         | 10.9          | 13.2    | 16.0  | 8.8   | 8.9    | 7.2   |
| Ash ...              | 1.6           | 2.8     | 2.2   | 1.8   | 1.6    | 0.9   |
| Loss, etc. ...       | 0.8           | 1.0     | 0.2   | 0.5   | 0.7    | 0.4   |
|                      | 100.0         | 100.0   | 100.0 | 100.0 | 100.0  | 100.0 |

Church gives the analyses undermentioned :—

|  | Fine<br>Wheat<br>Flour. | Rye<br>Flour. | Maize. | Millet. | Dari. |
|--|-------------------------|---------------|--------|---------|-------|
| Water ... ..                             | 13·0                    | 13·0          | 14·5   | 13·0    | 12·2  |
| Proteins ... ..                          | 10·5                    | 10·5          | 9·0    | 15·3    | 8·2   |
| Starch, with traces of<br>Dextrin ... .. | 74·3                    | 71·0          | 64·5   | 61·6    | 70·6  |
| Fat ... ..                               | 0·8                     | 1·6           | 5·0    | 5·0     | 4·2   |
| Cellulose and Lignin                     | 0·7                     | 2·3           | 5·0    | 3·5     | 3·1   |
| Mineral Matter ...                       | 0·7                     | 1·6           | 2·0    | 1·6     | 1·7   |
|  | 100·0                   | 100·0         | 100·0  | 100·0   | 100·0 |

### Composition of Maize and Rice Grits.

|                     | Maize<br>Grits. | Rice<br>Grits. |
|---------------------|-----------------|----------------|
| Starch ... ..       | 73·80           | 79·19          |
| Oil ... ..          | 0·82            | 0·80           |
| Proteins ... ..     | 9·05            | 8·91           |
| Mineral Matters ... | 0·40            | 0·30           |
| Moisture ... ..     | 10·85           | 10·30          |
| Cellulose, etc. ... | 5·08            | 0·50           |
|                     | 100·00          | 100·00         |

## Composition of Flaked Grains.

|                     | Rice.  | Maize. | Barley. |
|---------------------|--------|--------|---------|
| Starch ... ..       | 81·63  | 76·40  | 72·31   |
| Oil ... ..          | 0·20   | 1·30   | 1·72    |
| Proteins .. ..      | 8·73   | 9·82   | 10·35   |
| Mineral Matters ... | 0·37   | 0·45   | 2·35    |
| Moisture ... ..     | 7·90   | 7·50   | 5·82    |
| Cellulose, etc. ... | 1·17   | 4·53   | 7·45    |
|                     | 100·00 | 100·00 | 100·00  |

In the case of flaked maize, the germ and husk should have been specially removed prior to flaking. Barley is flaked without any such treatment, and rice, as received in this country, is usually already dehusked. In each flaked material, the whole of the starch granules are, or should be, ruptured.

## CHAPTER V.

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SUGARS.

THE varieties of sugars used in brewing may be divided into these classes :—

- I. Invert sugars.
- II. Starch saccharines.
- III. Cane sugars.

## I.—INVERT SUGARS.

The following determinations should be made :—

- Extract per cwt.
- Moisture.
- Invert sugar.
- Cane sugar.
- Nitrogen.
- Ash.
- Tests for iron, colour, etc.

## EXTRACT.

Carefully weigh out 25 grammes of the sugar to be analysed, dissolve in warm water, transfer to a 250 c.c. flask, wash in carefully, cool to 60° F., and make up to bulk. Take specific gravity of the solution at 60° F. To calculate the extract of the sugar multiply the excess weight over 1000 by 1.12.

The factor is thus obtained :—To reduce the excess weight over 1000 to lbs. per barrel multiply by 0·36. Now the above is a 10 per cent. solution, and each barrel of such solution contains, therefore, 36 lbs. of sugar, so  $112/36 = 3\cdot111$ , which factor gives extract per cwt. In place of using these two factors, we multiply them together and obtain factor 1·12 above given. Thus  $0\cdot36 \times 3\cdot111 = 1\cdot12$ .

*Example :—*

The specific gravity of a 10 per cent. solution was found to be 1032·0.

Then  $1032 - 1000 = 32$ ,

and  $32 \times 1\cdot12 = 35\cdot8$  lbs. extract per cwt.

## MOISTURE.

In the analysis of sugars, and similar substances, it is usual and preferable to determine the amount of water present by means of the specific gravity of the solution, and not by direct evaporation and drying of a portion of the sugar. The reason for this is that it is extremely difficult to remove the last traces of water from a sample without decomposing a portion of the sugar itself. To determine the amount of dry solids we divide the excess degrees over 1000 in specific gravity by 3·86.\* Thus the specific gravity of our 10 per cent. solution was 1032, and  $32 \div 3\cdot86 = 8\cdot29$  per cent. apparent solids in a 10 per cent. solution, or = 82·9 per cent. on the sugar. This result is, however, rendered inaccurate, and does not represent the true solids, by reason of the saline matter contained in the sugar sample, for whilst 1 gramme of pure sugar, when dissolved in 100 c.c. of water, gives approximately a specific gravity of

\* For explanation of this factor, see p. 101.

1003·86 (hence the factor), 1 gramme of the ash of sugar dissolved in water, and made up to 100 c.c., gives a specific gravity of 1008.

| Specific Gravity<br>10 per cent.<br>Solution. | Dry<br>Solids<br>Per cent. | Extract<br>Per cwt. | Specific Gravity<br>10 per cent.<br>Solution. | Dry<br>Solids<br>Per cent. | Extract<br>Per cwt. |
|---|----------------------------|---------------------|---|----------------------------|---------------------|
| 1030·0  | 77·72                      | 33·6                | 1032·0  | 82·90                      | 35·8                |
| ·1  | 77·97                      | 33·7                | ·1  | 83·15                      | 35·9                |
| ·2  | 78·23                      | 33·8                | ·2  | 83·41                      | 36·0                |
| ·3  | 78·50                      | 33·9                | ·3  | 83·67                      | 36·2                |
| ·4  | 78·75                      | 34·0                | ·4  | 83·93                      | 36·3                |
| ·5  | 79·01                      | 34·2                | ·5  | 84·20                      | 36·4                |
| ·6  | 79·27                      | 34·3                | ·6  | 84·45                      | 36·5                |
| ·7  | 79·53                      | 34·4                | ·7  | 84·71                      | 36·6                |
| ·8  | 79·79                      | 34·5                | ·8  | 84·97                      | 36·7                |
| ·9  | 80·05                      | 34·6                | ·9  | 85·23                      | 36·8                |
| 1031·0  | 80·31                      | 34·7                | 1033·0  | 85·50                      | 36·9                |
| ·1  | 80·57                      | 34·8                | ·1  | 85·75                      | 37·1                |
| ·2  | 80·82                      | 34·9                | ·2  | 86·01                      | 37·2                |
| ·3  | 81·08                      | 35·0                | ·3  | 86·26                      | 37·3                |
| ·4  | 81·35                      | 35·2                | ·4  | 86·53                      | 37·4                |
| ·5  | 81·60                      | 35·3                | ·5  | 86·78                      | 37·5                |
| ·6  | 81·86                      | 35·4                | ·6  | 87·04                      | 37·6                |
| ·7  | 82·12                      | 35·5                | ·7  | 87·30                      | 37·7                |
| ·8  | 82·38                      | 35·6                | ·8  | 87·56                      | 37·8                |
| ·9  | 82·64                      | 35·7                | ·9  | 87·82                      | 37·9                |
|   |                            |                     | 1034·0  | 88·08                      | 38·1                |

In order, therefore, to be accurate, we ought to divide that portion of specific gravity due to sugar by 3·86, and that due to mineral matter by 8. Now as we know the amount of mineral matter present\* we can either deduct

\* See p. 201.

the gravity that may be due to this, or, after calculating the apparent solids by the use of the 3.86 factor, make the following correction for the disturbing influence of the mineral matter.

Since the mineral matter is expressed too high as dry solids in the ratio of 8 to 3.86, multiply the ash by 8, and divide by 3.86, or use the factor 2.07 ( $\frac{8}{3.86} = 2.07$ ), then deduct the result so found from the apparent solids, which gives the solids due to actual organic matter, thus:—

$$2.03 \text{ (ash)} \times 2.07 = 4.20,$$

and  $82.90 \text{ (apparent solids)} - 4.20 = 78.70$  solids due to organic matter. If we now add to this figure the amount of actual ash (2.03) we obtain

$$78.70 + 2.03 = 80.73,$$

the true total amount of solid matter per cent. in the sugar, and this deducted from 100 gives the moisture percentage, viz., 19.27.

## INVERT SUGAR.

Two methods are described—one a volumetric and the other a gravimetric process. The volumetric method is not recommended, for although it is fairly accurate with solutions of absolutely pure invert sugar, yet with commercial samples it yields results considerably below the truth. The method is given as it is very easily conducted, and affords a rapid and rough valuation of the sample, but, wherever possible, the gravimetric method should be adopted. The authors always carry out a volumetric



estimation as a preliminary to the gravimetric, for reasons hereafter stated, and the result obtained volumetrically is also used in the part determination of the cane sugar (*q.v.*).

Both volumetric and gravimetric methods depend upon the fact that a mixture of copper sulphate, potassium tartrate, and caustic soda in certain proportions, known as "Fehling's solution," may be boiled without undergoing any change, but if only a trace of a "reducing" sugar be added a slight warming suffices to precipitate a portion of the copper as red cuprous oxide,  $\text{Cu}_2\text{O}$ , and working under certain standard conditions the amount so precipitated is in constant proportion to the quantity of sugar added, thus enabling methods to be devised for sugar estimation.

Reducing sugars belong to a class of substances which have a powerful affinity for oxygen, and which can, under appropriate conditions, reduce many of the metallic oxides to a lower oxide or to the metal.

The copper salt of Fehling's solution is reduced to one of the lower oxides of copper and not to the metal itself.

In the volumetric process a measured volume of the standard Fehling's solution is taken, and the sugar-containing solution added until the whole of the copper salt is just reduced to its oxide. Then, the reducing power of the sugar being known, its percentage in the solution under examination can be easily calculated.

Much depends upon the dilution of the Fehling's solution, and when using the factors here given *it is necessary to carefully observe the conditions of working as described.*

In the gravimetric process the copper sulphate is left in excess after its part reduction by the sugar solution and the reducing power of the sugar is known when the weight of copper oxide so obtained is within certain limits and the method carried out under approximately standard conditions.

The dilution of the Fehling, length of time of boil, size of beaker, and other conditions, all influence the amount of precipitated copper, so that with this method it is quite as necessary to adhere rigidly to the conditions laid down as with the volumetric one.

When determining sugar by either method, the same Fehling's solution is used, but in the gravimetric no exact standardising is necessary: it is only required to weigh out the quantities as hereafter given.

Formerly it was the custom to keep the Fehling's solution ready prepared, but it was found to deteriorate very rapidly and became worthless in a few weeks. This was a very serious drawback, as the trouble of preparing an accurately adjusted solution is considerable. If, however, the cupric sulphate solution is kept separate from the mixture of Rochelle salt and caustic soda, the former keeps for an almost indefinite length of time, especially if the solution is slightly acid; while the latter solution, although not so permanent as the former, remains unchanged for a considerable period, and even when partially decomposed can without trouble be replaced by a fresh solution, as no particular accuracy is needed in its preparation, but it is most important that the chemicals employed be perfectly pure.

Fehling's solution may be purchased ready standardised, but it is very much more satisfactory for the student to prepare and standardise the solutions himself, as only under these conditions can subsequent estimations be made on strictly comparative lines with those used in the standardisation, an essential condition for obtaining accurate results.

The separate solutions are prepared as follows:—

**Standard Fehling's Solution.**—*Alkaline Tartrate Preparation.*—Weigh out 350 grammes of Rochelle salt and 100 grammes of caustic soda—the purity of which is essential and that termed “pure by alcohol” is best. Dissolve in distilled water with the aid of heat, cool, and dilute to 1 litre. Should the solution be cloudy, as is sometimes the case, it may be filtered into the store bottle through a funnel containing a plug of glass wool.

*Copper Solution: Preparation and Standardisation.*—If perfectly pure and dry crystals of cupric sulphate can be obtained, a solution of exactly the correct strength is prepared by dissolving 69.28 grammes in 1 litre of distilled water; but as this is very seldom the case, it is advisable to weigh up about 72 grammes, which will produce a solution somewhat over the correct strength. The copper sulphate after being weighed out is dissolved in hot distilled water, cooled, and 4 c.c. of strong, pure sulphuric acid added. The solution is again cooled and made up to 1 litre at 60° F. It is standardised and corrected as follows, and in effecting this the greatest accuracy is requisite, as a small difference in the strength of the copper solution causes a considerable error when calculated out into sugar percentage in the ordinary manner.

It is necessary to prepare a standard solution of inverted sugar, and to do this we proceed as follows:—

The purest cane sugar obtainable is weighed out, making such allowance for moisture as has been found necessary in a determination either by drying in a water bath, or, still better, by the polarimeter. The variety known as “coffee sugar” has been found to be the purest commonly obtainable; indeed, it generally contains from 99.2 to 99.8 per cent. of absolute cane sugar, and if the larger crystals are selected it contains absolutely no foreign substance.

Two grammes of actual sugar are weighed out and placed in a

scrupulously clean 200 c.c. flask with 50 c.c. of water, 5 c.c. of 4N acid\* added, and the whole heated in an inversion bath at 150° F. for 20 minutes. At the expiration of this time the flask is taken out, cooled, and 5 c.c. of 4N alkali added; the bulk being afterwards made up to exactly 200 c.c. at 60° F.

A burette is filled with this solution and a Fehling conducted in exactly the same way as described for the volumetric estimation of invert sugar in the next paragraph, the exact quantity of such solution required being accurately determined. Having found the quantity, and knowing by calculation what quantity correct Fehling should require, we can easily ascertain the dilution necessary to bring the copper solution to proper strength. The calculation will be clearly seen if an example is worked out.

The coffee sugar taken was found—by polarimeter—to contain 99.8 per cent. of real cane sugar; 2 grammes of actual sugar was required, so that  $2.004$  ( $99.8 : 100 :: 2 = 2.004$ ) grammes was weighed out, inverted with 5 c.c. 4N acid, neutralised, and diluted to 200 c.c.

To ascertain how many cubic centimetres of this the copper solution should take—first, calculate gain of cane sugar on inversion, thus:— $95 : 100 :: 2 = 2.10$  grammes invert sugar in 200 c.c., and  $2.10 \div 2 = 1.05$  in 100 c.c. Now if 100 c.c. contain 1.05 grammes of invert sugar, how many cubic centimetres will contain 0.25 gramme—the quantity corresponding to 50 c.c. of Fehling?

$1.05 : 100 :: 0.25 = 23.8$  c.c. of the sugar solution should be required to reduce 50 c.c. of Fehling.

But it was found that 30.1 c.c. was used, so that the Fehling solution is too strong. What factor would 30.1 c.c. correspond to?

$23.8 : 0.25 :: 30.1 = 0.316$ ; therefore, each 50 c.c. of Fehling, or 25 c.c. of copper solution, equals 0.316 invert sugar instead of 0.25. How many cubic centimetres of correct standard copper solution should represent 0.316 gramme?

If 25 c.c. equals 0.25, how many cubic centimetres does 0.316 equal?

Factor, c.c.

$$0.25 : 25 :: 0.316 = 31.6 \text{ c.c.}$$

So that if we dilute 25 c.c. of our copper solution to 31.6 c.c. it will be of exactly the proper strength.

Or, add to each 25 c.c. 6.6 c.c. of distilled water, or to each litre 264 c.c.

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\* For preparation of 4N acid and alkali, see p. 412.

This was done, and another Fehling conducted after perfect admixture. It required 23·8 c.c. and was therefore correct.

**Volumetric Estimation.**—Twenty cubic centimetres of the 10 per cent. solution of sugar is measured into a 200 c.c. flask, diluted with water to the mark, and well mixed (= 1 per cent. solution). A burette is filled with this liquid, rinsing it once or twice with the solution prior to filling. Now prepare the Fehling's solution as follows :—

200 c.c. of distilled water is placed in a boiling flask of 700 or 800 c.c. capacity, and raised to the boiling point. When well boiled, 50 c.c. of Fehling's solution is added, that is 25 c.c. of standard cupric sulphate, and 25 c.c. of alkaline tartrate. On first contact of these solutions, a precipitate frequently forms, which entirely redissolves on mixing, when the solution should be perfectly brilliant, and of a rich deep blue colour. Any cloudiness or light colour indicates the decomposition of one or both of the solutions. (The Fehling prepared as thus directed is, it will be seen, diluted with four times its bulk of water. It is always necessary to work with this dilution, for the reducing powers of the sugars vary with the dilution of the Fehling, and all the factors for Fehling's solution here given are based on the supposition that it has been so diluted.) The contents of the flask are now again raised to boiling point, some pieces of finely drawn out glass tubing added to prevent bumping, and 10 c.c. of the sugar solution run in from the burette. The whole is again boiled for two minutes, then further quantities of the sugar solution added at the rate of 5 c.c. at a time, the boiling being

## Volumetric Process for Sugar Determination. Factor .25.

| 1 per cent.<br>Sugar Solution. | Invert Sugar. | 1 per cent.<br>Sugar Solution. | Invert Sugar. |
|--------------------------------|---------------|--------------------------------|---------------|
| c.c.                           | Per cent.     | c.c.                           | Per cent.     |
| 29·0                           | 86·20         | 32·6                           | 76·69         |
| ·1                             | 85·91         | ·7                             | 76·45         |
| ·2                             | 85·61         | ·8                             | 76·22         |
| ·3                             | 85·32         | ·9                             | 75·99         |
| ·4                             | 85·03         | 33·0                           | 75·76         |
| ·5                             | 84·74         | ·1                             | 75·53         |
| ·6                             | 84·46         | ·2                             | 75·30         |
| ·7                             | 84·17         | ·3                             | 75·07         |
| ·8                             | 83·89         | ·4                             | 74·85         |
| ·9                             | 83·61         | ·5                             | 74·62         |
| 30·0                           | 83·33         | ·6                             | 74·40         |
| ·1                             | 83·05         | ·7                             | 74·18         |
| ·2                             | 82·78         | ·8                             | 73·96         |
| ·3                             | 82·51         | ·9                             | 73·74         |
| ·4                             | 82·23         | 34·0                           | 73·53         |
| ·5                             | 81·96         | ·1                             | 73·31         |
| ·6                             | 81·69         | ·2                             | 73·10         |
| ·7                             | 81·43         | ·3                             | 72·89         |
| ·8                             | 81·17         | ·4                             | 72·67         |
| ·9                             | 80·90         | ·5                             | 72·46         |
| 31·0                           | 80·64         | ·6                             | 72·25         |
| ·1                             | 80·38         | ·7                             | 72·05         |
| ·2                             | 80·12         | ·8                             | 71·84         |
| ·3                             | 79·87         | ·9                             | 71·63         |
| ·4                             | 79·61         | 35·0                           | 71·43         |
| ·5                             | 79·36         | ·1                             | 71·22         |
| ·6                             | 79·11         | ·2                             | 71·02         |
| ·7                             | 78·86         | ·3                             | 70·82         |
| ·8                             | 78·61         | ·4                             | 70·62         |
| ·9                             | 78·37         | ·5                             | 70·42         |
| 32·0                           | 78·12         | ·6                             | 70·22         |
| ·1                             | 77·88         | ·7                             | 70·03         |
| ·2                             | 77·64         | ·8                             | 69·83         |
| ·3                             | 77·40         | ·9                             | 69·64         |
| ·4                             | 77·16         | 36·0                           | 69·44         |
| ·5                             | 76·92         |                                |               |



continued after each addition. The blue colour of Fehling becomes paler as an increasing quantity of sugar solution is run in, a bright red precipitate of cuprous oxide ( $\text{Cu}_2\text{O}$ ) being formed. When the blue colour seems to be nearly discharged, the solution is added more cautiously, 1 c.c. at a time, and afterwards, when the final point is nearly reached, in quantities of half a cubic centimetre or even less. When it is thought that the reduction is accomplished, and with a little experience the peculiar boiling is a useful indication, the flask is taken off the flame, and the precipitate allowed to settle. It is then easy to see if any trace of blue colour remains, and the end of the reaction may be ascertained by this means with fair accuracy. In order, however, to be assured as to the complete reduction and consequent removal of the copper from solution, it is always advisable to apply a test to the liquid. A filter of small size is prepared, and moistened with warm—preferably boiling—water, and a little of the hot Fehling liquid is filtered and collected in a small test-tube. Having ascertained that the filtrate is perfectly bright, and free from the red cuprous oxide, it is tested for copper by adding a few drops of acetic acid (to neutralise the alkali and render the solution *very* slightly acid), and one drop of solution of ferrocyanide of potassium. If there be any copper, a reddish-brown coloration will be produced, varying in intensity with the quantity present. Should, however, there be but a *faint* bronze tint, it is an indication that only the merest trace of copper is still remaining in solution, and that the experiment is complete. If the test shows the presence of copper, a further addition of the sugar solution



is made, and the contents of flask again boiled and tested. This is repeated until, by the appearance, on testing, of only a very slight bronze tint, the reduction is found to be complete. In testing, it is essential that both the filter and liquid should be hot, otherwise some of the cuprous oxide may re-enter into solution, and thus vitiate the results. It is also always advisable to test before it is thought that the reaction is quite completed, as it is sometimes difficult to tell if it be overdone, but absolutely easy to ascertain the reverse. If this point has been overstepped, the tested liquid will appear perfectly yellow and opalescent after the addition of the ferrocyanide, in which case it is, of course, necessary to repeat the estimation from the beginning.

It must also be recollected that each withdrawal of a portion of the solution for the purpose of testing really weakens the strength of that remaining, by abstracting a certain quantity of unreduced copper. Should, therefore, more than two withdrawals have been made, the estimation should be repeated in all cases where accuracy is required. No portion which has been withdrawn, and has had either acetic acid or potassic ferrocyanide added, must in any case be returned to the flask, as it would entirely spoil the experiment.

The reduction being completed, the number of cubic centimetres required is read off the burette, and since 50 c.c. of Fehling requires 0.25 gramme of invert sugar to reduce it, the volume of sugar solution added must contain that quantity of invert sugar, and a simple calculation gives the percentage of invert sugar in the sample.

*Example :—*

Using a 1 per cent. solution of sugar, and 50 c.c. of Fehling, 34.2 c.c. was required to complete the reduction.

Then  $0.25 \times \frac{100}{34.2} = 0.731$  gramme of invert in 100 c.c.

of 1 per cent. solution, or 1 gramme of sugar,

and  $0.731 \times 100 = 73.1$  per cent. of invert sugar in sample.

Ling and Rendle\* have suggested the use of ferrous thiocyanate as an indicator in place of the ferrocyanide of potassium. It is prepared by dissolving 1 gramme of ferrous ammonium sulphate and 1 gramme of ammonium thiocyanate in 10 c.c. of warm water, cooling immediately the salts have dissolved. Five cubic centimetres of concentrated hydrochloric acid are then added.

The red colour rapidly developed by the solution is decolorised by a trace of zinc dust, but after several treatments the indicator loses its sensitiveness.

A drop of the indicator mixed with a drop of the copper solution on a white slab will give an immediate red coloration so long as there is a trace of unreduced copper present. The indicator is not available, however, when iron is present.

In a further paper† they show that the presence of cane sugar does not affect the accuracy of the invert sugar estimation unless the cane sugar exceeds 30 per cent. of the total sugars present.

**Gravimetric Estimation.**—As before mentioned, this method should always be adopted where accuracy is required. Proceed as follows :—

Measure 50 c.c. of distilled water into a thin white porcelain beaker, 3 inches in height, 2 inches in diameter, and of about 150 c.c. capacity. Add 30 c.c. of Fehling's solution, *i.e.*, 15 c.c. each of copper sulphate solution and alkaline tartrate solution. Just raise to the boil, remove

\* *Analyst*, 1905, p. 182.

† *Analyst*, 1908, p. 167.

the beaker, and immediately run into it the prepared 1 per cent. sugar solution from a burette. The quantity of sugar solution added must be so regulated that the weight of  $\text{CuO}$  obtained lies between 0.27 and 0.30 grammes. Unless this is arranged, the result will not be accurate when using factors here given. The volumetric estimation will have enabled us to calculate the amount of the 1 per cent. solution of the sugar which it will be necessary to add to bring the quantity of copper oxide within these limits. If in the volumetric test about 33 c.c. has been taken, the quantity of sugar solution (1 per cent.) to be employed in our gravimetric test should be 18 c.c. If, on the other hand, about 35 c.c. or 30 c.c. has been used, then the amounts of sugar solution to be employed are 19 c.c. and 17 c.c. respectively. Now replace the beaker on the wire gauze over the Bunsen burner, mix the contents by stirring with a small glass rod, afterwards withdrawing this, first rinsing it clean with a few drops of hot distilled water from the wash bottle, which latter should be kept ready filled with boiling water.

Cover the beaker with a small clock glass, and again just raise to the boil, immediately removing the burner when this takes place.

Stand the beaker in boiling water for 10 minutes, then filter through a good filter paper.

After decanting off all the blue liquid, carefully wash the red oxide of copper precipitate into the paper as quickly as possible with hot distilled water, taking care that every particle is in this way removed from the beaker. Do not allow the precipitate to dry on to the bottom or sides of the

beaker during the process of filtration, or considerable difficulty will be found in afterwards detaching it; the process of transferring the copper oxide from the beaker into the filter paper must be rapid. A sound filter paper, free from minute pin-holes, etc., must be used, as otherwise some of the precipitate is very liable to pass through. If any has done so, it will, after standing, be found on the bottom of the beaker containing the filtrate, in which case the liquid is poured off, and the precipitate returned to the filter.

When the whole of the oxide has been collected on the filter paper in this way, wash it several times with hot water by decantation—do not blow a stream of hot water from the wash bottle directly on the filter paper.

When all trace of copper has been washed out—ascertained by testing the final washings with a few drops of potassium ferrocyanide solution—dry the filter in a hot water jacket, and transfer the precipitate as far as possible into a platinum crucible—previously heated, cleaned, and weighed.

Now burn off the paper, as described on p. 39, adding this to the oxide already in the crucible, and very gently heat the crucible without its lid for a few minutes over a Bunsen flame. Care must be taken to ignite carefully at first, as otherwise some cuprous oxide is liable to become coated with an outer layer of cupric oxide, and so prevent complete oxidation.

Finally, heat strongly to redness for 10 minutes, then cool and weigh. Repeat this, if necessary, until the weight is constant.

The copper now exists as black oxide, the red cuprous form  $\text{Cu}_2\text{O}$  having been oxidised to the black cupric  $\text{CuO}$ .

Calculate the invert sugar from the copper oxide obtained, each gramme of  $\text{CuO}$  being equivalent to 0·4715 grammes of invert sugar.

*Example :—*

18 c.c. of 1 per cent. solution of sugar gave 0·283 gramme of  $\text{CuO}$ .

Then  $0\cdot283 \times 0\cdot4715 = 0\cdot1334$  invert sugar in 18 c.c.,  
and  $0\cdot1334 \times \frac{100}{18} = 0\cdot7411$  invert sugar in 100 c.c. of 1 per cent. solution, or 1 gramme of sugar,  
= 74·11 per cent. on sample.

Using 18 c.c. of 1 per cent. solution,  $\text{CuO}$  found equals sugar as under (factor ·262) :—

|            | Per cent. |            | Per cent. |
|------------|-----------|------------|-----------|
| ·300 ..... | 78·60     | ·287 ..... | 75·20     |
| ·299 ..... | 78·35     | ·286 ..... | 74·95     |
| ·298 ..... | 78·05     | ·285 ..... | 74·65     |
| ·297 ..... | 77·80     | ·284 ..... | 74·40     |
| ·296 ..... | 77·55     | ·283 ..... | 74·15     |
| ·295 ..... | 77·30     | ·282 ..... | 73·90     |
| ·294 ..... | 77·00     | ·281 ..... | 73·60     |
| ·293 ..... | 76·75     | ·280 ..... | 73·35     |
| ·292 ..... | 76·50     | ·279 ..... | 73·10     |
| ·291 ..... | 76·25     | ·278 ..... | 72·85     |
| ·290 ..... | 76·00     | ·277 ..... | 72·55     |
| ·289 ..... | 75·70     | ·276 ..... | 72·30     |
| ·288 ..... | 75·45     | ·275 ..... | 72·05     |

|            | Per cent. |            | Per cent. |
|------------|-----------|------------|-----------|
| ·274 ..... | 71·80     | ·266 ..... | 69·70     |
| ·273 ..... | 71·50     | ·265 ..... | 69·45     |
| ·272 ..... | 71·25     | ·264 ..... | 69·15     |
| ·271 ..... | 71·00     | ·263 ..... | 68·80     |
| ·270 ..... | 70·75     | ·262 ..... | 68·65     |
| ·269 ..... | 70·45     | ·261 ..... | 68·40     |
| ·268 ..... | 70·20     | ·260 ..... | 68·10     |
| ·267 ..... | 69·95     |            |           |

In making this estimation, the foregoing instructions must be rigidly adhered to. Differences in the dilution of the Fehling's solution, the relative amount of sugar to copper, and the length of time of heating, each alter the quantity of copper precipitated. The estimation must be done in duplicate, and the two results should agree within 1 or 2 milligrammes.

Traces of Fehling's solution are retained by the filter paper, and are not removed even by continued washing, so that a special correction must be made for the ash of the filter paper when using a copper solution in this way.

This is usually found to be 0·006 grammes, but the ash of any particular paper used can readily be determined by making a blank experiment, following the gravimetric process in detail with about half a dozen papers, burning these off, weighing them together, and so obtaining an average correction for a single paper to be deducted from the total weight of copper oxide obtained in each sugar determination.

With care, very accurate results can be obtained by weighing the copper as cupric oxide in the way described, but some chemists prefer to filter the red cuprous oxide through asbestos contained in a special crucible, afterwards reducing to metallic copper by heating in a current of hydrogen and weighing it in the metallic state.

Others again prefer to weigh the oxide at once as cuprous oxide, but this method cannot be recommended.

**Determination of Dextrose and Levulose.**—It is sometimes desirable not only to estimate the total reducing sugars and express them as invert sugar but to calculate also the percentages of each of its components, namely, dextrose and levulose.



Theoretically, equal weights of each of these two substances result from the inversion of a cane sugar, but practically there is often considerable difference in the relative percentages, and levulose, being more easily decomposed by the inversion treatment than dextrose, is obtained in lower relative percentage. This difference varies from 6—12 per cent., and the percentages of these two sugars in a sample of invert gives some indication as to the mode of manufacture—the greater the destruction of levulose the more severe has been the inversion process, this being also to some extent an indication of the particular grade of raw sugar used.

The process is described by Morris in the *Journ. Inst. Brew.*, 1898, p. 162, but the method is not an easy one to carry out, and sometimes gives results of doubtful value.

## CANE SUGAR.

Measure 20 c.c. of the 10 per cent. solution of sugar, previously prepared (see p. 182), into a 200 c.c. flask, with 50 c.c. of water and 5 c.c. 4N hydrochloric acid.\* Keep the flask in a bath of hot water at 150° F. for 20 minutes. Then remove, cool to 60° F., neutralise by the addition of 5 c.c. 4N alkali, and dilute to mark and mix. We have now a 1 per cent. solution of the sample, containing the invert sugar originally present, plus that produced from inversion of cane sugar. The increase of invert sugar represents, therefore, the proportion of cane sugar in the sample. Determine the cupric oxide reducing power as before, calculate the amount of total invert sugar in the sample, and multiply the increase thus found by 0.95, which gives the cane sugar present in the sample.

*Example :—*

18 c.c. of 1 per cent. inverted solution of sugar gave 0.291 gramme of CuO.

\* For preparation, see p. 412.



Then  $0.291 \times 0.4715 = 0.1372$  total invert in 18 c.c.,  
 and  $0.1372 \times \frac{100}{18} = 0.7622$  total invert in 100 c.c. of 1 per  
 cent. solution, or 1 gramme of sugar,  
 $= 76.22$  per cent. of total invert in sample.

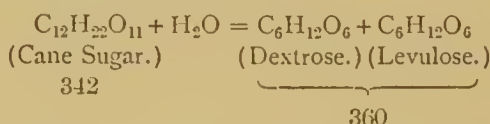
Then  $76.22 - 74.11$  (the actual invert found in first  
 experiment)  $= 2.11$  invert due to cane, and

$2.11 \times 0.95 = 2.00$  per cent. of cane sugar.

The explanation of the factor 0.95 is as follows :—

Cane sugar has the formula  $C_{12}H_{22}O_{11}$ , and by inversion it is trans-  
 formed into an equal mixture of the two sugars, dextrose and levulose,  
 $C_{12}H_{24}O_{12}$ , and is termed *invert sugar*.

An equation renders this change clear :—



So that 360 parts of invert sugar are produced from 342 parts of  
 cane sugar, and a simple calculation gives the cane sugar corresponding  
 to each 100 parts of invert sugar :—

$$360 : 342 :: 100 = 95.$$

**Yeast Inversion.**—In place of acid, yeast may be used for the  
 inversion as follows :—

20 c.c. of the 10 per cent. solution of sugar is measured into a  
 200 c.c. flask, about 40 c.c. of water, and from 0.5 to 1 gramme of  
 pressed yeast added. The flask is then placed in a water bath, and  
 kept at 130° F. for five hours, when the inversion should be complete.  
 The solution is now cooled to 60° F., a little aluminic hydrate added,  
 and the whole made up to bulk (200 c.c.) and mixed, then filtered  
 bright, and the sugar estimated in the usual way.

## NITROGEN.

It is often unnecessary to determine this, but if required 4 grammes of the sample are taken, and the nitrogen estimated by Kjeldahl's process, as already described on p. 119.

Many sugars break down only with considerable difficulty, and using a less quantity than 4 grammes will accelerate matters, but this at the same time increases the margin of error.

*Example :—*

4 grammes of the sugar required 3.9 c.c. of N/10 alkali to neutralise the ammonia from the protein substance.

Then  $3.9 \text{ c.c.} \times 0.009 = 0.0351$  protein in 4 grammes sugar, and  $0.0351 \times \frac{100}{4} = 0.88$  per cent. protein in the sample.

## ASH.

About 4 grammes of sugar are weighed into a platinum dish and cautiously ignited. At first a very small flame must be used owing to the great swelling of the semi-carbonated mass. Afterwards the ash shrinks, and the dish may then be covered loosely with a piece of platinum foil, which will much accelerate the oxidation of the carbonaceous matter. In the case of stubborn ashes, which persistently refuse to burn white, it is well to take away the flame, allow the dish to cool, add a few drops of a saturated solution of ammonium nitrate, and again apply heat, the nitrate oxidising the carbon. Sometimes, also, it

is advisable to "sulphate" an ash, that is, to add a little strong sulphuric acid, thereby converting the whole of the salts into sulphates. When this is done the operation is best accomplished by adding a few drops of sulphuric acid to the sugar before commencing to burn off.

When no more carbon remains, and the ash is either white, light grey, or brown—this latter in the presence of iron—it is placed under a desiccator, and, when cool, weighed. The ash is then dissolved out by means of hydrochloric acid, the dish carefully cleaned, ignited, and re-tared. This is necessary as, if the ignition has been at all prolonged, the dish may probably have lost in weight. The results so obtained may be easily calculated to a percentage. The colour of the ash should be carefully observed, and if brown or reddish should be dissolved in pure hydrochloric acid and tested for iron.

*Example :—*

4.136 grammes of sugar gave an ash weighing 0.084 gramme.

$$\text{Then } 0.084 \times \frac{100}{4.136} = 2.03 \text{ per cent. of ash.}$$

Formerly it was usual when an ash had been sulphated to deduct one-tenth of the weight found, but as it is probable that the actual weight of the sulphated ash represents with fair accuracy the actual amount of mineral matter present in the sugar, this deduction is not, as a rule, now made.

## OTHER BODIES.

The sum of the foregoing determinations (not, of course, including the extract per cwt.) will not as a rule amount to 100. The difference thus found is often (it was at one

time invariably) stated as "inert matter." Most commercial sugars contain a certain amount of carbohydrates other than invert and cane sugar. These are probably the products of over-inversion, and some of them exert a reducing action on Fehling, though lower than that of invert sugar. It is, therefore, probable that we are stating our invert sugar proportion too high, for we are expressing the whole of the copper reduced as invert, whereas a portion is due to other carbohydrates, whilst by expressing our invert sugar above, we are also expressing the other carbohydrates correspondingly below the truth. At the present time, however, we have no very satisfactory means of ascertaining the precise amount of these bodies, though probably something may be done by fermenting away the invert sugar, and determining the total weight, opticity, and reducing power of the residue. Such a method is not yet, however, sufficiently satisfactorily worked out to permit of its inclusion here.

The amount of "other bodies" is, therefore, found as under:—

|                                  |     |     |                 |
|----------------------------------|-----|-----|-----------------|
| Total solids in sugar ...        | ... | ... | 80.73 per cent. |
| Less bodies determined           | ... | }   | 79.02 „         |
| (Invert + cane + N matter + ash) |     |     |                 |
| Other bodies                     | ... | ... | 1.71 „          |

## OPTICAL ACTIVITY.\*

It is always desirable to determine this, not in most cases for the purpose of direct use in the analysis, but in order to detect the addition of starch glucose, if such has been made.

The optical activity of an invert sugar should be lævoro-rotatory. If dextro-rotatory, then the sugar either contains much cane sugar, or there is present an admixture of starch glucose, and the amount of this may be calculated from the optical activity. Whilst, however, invert sugar should theoretically consist of equal weights of dextrose and levulose, commercial samples always contain an excess of dextrose, owing to some decomposition of levulose during inversion as already mentioned. Allowance for this fact must be made if attempting to estimate the added starch glucose.

In stating optical activity, it must be remembered that this varies with temperature. The determinations should be made strictly at 60° F.

To carry out experiment, polarise a 10 per cent. solution at 60° F. in 100 mm. tube.

*Example :—*

Reading gave  $-1.235 [\alpha]_D = -12.35 [\alpha]_D$  on sugar.

## IRON.

This cannot be tested for in the sugar solution directly ; a satisfactory examination for this substance can only be made by dissolving the ash in acid, testing this with

\* For description of the Polarimeter, see p. 400.

potassium ferrocyanide solution, and comparing the colour so obtained with that given by an approximately similar volume of a standard iron solution.

The estimation may be made as follows :—

The percentage of ash having been obtained, it is dissolved in a few drops of pure concentrated hydrochloric acid—free from iron—and evaporated down to dryness on the water bath, and again dissolved in dilute hydrochloric acid of approximately known strength.

A little bromine water is added to oxidise any ferrous salts to ferric, and the liquid boiled to expel excess of bromine, cooled, and made up to the mark in a 100 c.c. flask.

*A standard solution of iron* is now prepared as follows :—

Weigh out 0·7 gramme of ferrous ammonium sulphate, and dissolve in about 250 c.c. of distilled water, add bromine water until the liquid remains permanently yellow, boil off the excess of bromine with the addition of a few drops of dilute hydrochloric acid, cool, transfer to a litre flask, and dilute to the mark.

*Each 1 c.c. of this solution then equals 0·1 milligramme of iron.*

Take 5 c.c. of this solution, and make up to 100 c.c.

We now have our sugar and standard solutions ready for the estimation ; 50 c.c. of each are taken in a Nessler test-tube, and a few drops of potassium ferrocyanide solution added. After standing for a minute the intensity of blue colour in each is compared, and fresh measures of the standard solution must be taken if necessary, until the

colours of the same volumes of liquid are as nearly as possible alike.

In the determination of ammonia in water analysis (see p. 253), it is sufficiently accurate to obtain comparative colours by altering the depth, or volume, of the standard solution, but considerable error would be introduced if this plan were adopted in iron estimation, and accurate results can only be obtained by comparing approximately equal volumes.

*Example*.—Suppose the tubes were of equal colour when 50 c.c. of the sugar ash solution was compared with 50 c.c. of the diluted standard iron solution. In this case 50 c.c. of the ash solution represents 2.068 grammes of sugar (4.136 grammes being taken for the determination of ash percentage), and 50 c.c. of diluted standard = 0.00025 gramme of iron,

therefore,  $\frac{100 \times 0.00025}{2.068} = 0.012$  per cent. iron, Fe, or

0.017 per cent. expressed as oxide of iron,  $\text{Fe}_2\text{O}_3$ .

The colours are compared as described in the estimation of ammonia under "water analysis" by looking down the Nessler tube raised slightly over a white slab or paper. It is advisable to oxidise the standard iron solution each time this is used with a little bromine water as above described, and further, the amount of acid used for dissolving the ash should be approximately the same as that added to the standard solution when re-oxidising, for, unless strictly comparative conditions are observed, it is difficult to get colour readings of the same blue shade.

## COLOUR, ETC.

The colour of the sugar should be registered by means of the tintometer. Determine this in a 10 per cent. solution



by Lovibond's tintometer, using a 1-inch cell, as described under "Malt Analysis," and express the colour on the sugar.

*Example :—*

Reading was  $8.5^\circ$ , using the 52 series of glasses =  $85^\circ$  on the sugar.

Test the *reaction* with litmus paper. Occasionally some inverts are purposely left faintly acid, as they are then of a paler colour. If necessary the acidity may be determined on 100 c.c. of the 10 per cent. solution, titrating with N/10 alkali. Observe the flavour of the sugar, the brilliancy or otherwise of its solution, and note whether any precipitate is thrown down when the solution is boiled, which ought not to be the case.

The completed analysis as above gives the following figures :—

|                      |     |     |     | Per cent.           |
|----------------------|-----|-----|-----|---------------------|
| Invert sugar         | ... | ... | ... | 74.11               |
| Cane sugar           | ... | ... | ... | 2.00                |
| Mineral matter       | ... | ... | ... | 2.03                |
| Nitrogenous matter   | ... | ... | ... | 0.88                |
| Moisture             | ... | ... | ... | 19.27               |
| Other bodies, etc.   | ... | ... | ... | 1.71                |
|                      |     |     |     | <hr/> 100.00 <hr/>  |
| Optical activity     | ... | ... | ... | $-12.35 [\alpha]_D$ |
| Extract per 112 lbs. | ... | ... | ... | 35.84 lbs.          |
| Colour               | ... | ... | ... | $85^\circ$          |

## II.—STARCH SACCHARINES.

The starch saccharines consist of maltose and dextrose (glucose) and a certain proportion of dextrin, unfermentable bodies, etc.

The following determinations should be made :—

Extract.

Cupric oxide reducing power.

Cupric oxide reducing power after fermentation.

Optical activity.

Optical activity after fermentation.

Nitrogen.

Ash.

Moisture.

Test for iron, colour.

### EXTRACT.

This is determined precisely as in an invert sugar.

*Example :—*

Specific gravity of a 20 per cent. solution = 1067°, and  
 $67 \times 0.56 = 37.52$  lbs. extract per cwt.

### CUPRIC OXIDE REDUCING POWER.

First determine the approximate reducing power of the sugar on a 1 per cent. solution, by the volumetric process, as previously described. Then, having by that means ascertained the correct quantity of the 1 per cent. solution to be used, carry out a gravimetric estimation, and

calculate the cupric oxide obtained to dextrose by the use of the factor 0.4535.

*Example:—*

17 c.c. of a 1 per cent. solution gave 0.294 cupric oxide, and  $0.294 \times \frac{100}{17} = 1.73$  cupric oxide from 1 gramme of sugar.

$$1.73 \times 100 = 173 \text{ CuO per cent.}$$

$173 \times 0.4535 = 78.45$ , total reducing sugars expressed as dextrose.

### OPTICAL ACTIVITY.

Determine in 10 per cent. solution with 200 mm. tube, or—if the colour is too deep—with 100 mm. tube.

*Example:—*

Reading obtained in 200 mm. tube =  $+5^\circ$

$$+5^\circ \times 10 = +50^\circ [\alpha]_D \text{ on sugar.}$$

### REDUCING POWER AND OPTICAL ACTIVITY AFTER FERMENTATION.

Take 100 c.c. of 20 per cent. solution, place in a flask, add about 50 c.c. of distilled water and 10 c.c. of yeast water, prepared by mixing about 1 part of pressed yeast with 10 parts of water, and boiling the solution for a few minutes. This is then cooled, filtered bright, and the bright filtrate used. Now add 1 gramme of washed and pressed brewers' yeast. Loosely plug the mouth of flask with cotton wool, and place on forcing tray. Keep at a temperature of  $75^\circ$  to  $80^\circ$  F. Leave till fermentation is complete, boil off the alcohol, then cool; transfer, and

wash into 200 c.c. flask, add a few drops of aluminic hydrate, make up to mark, mix well, and filter. Use the bright filtrate. Determine reducing power on 20 c.c. (in some cases 10 to 15 c.c. must be used) of the 10 per cent. solution thus obtained, and calculate to dextrose.

*Example :—*

20 c.c. of fermented solution gave 0.296 gramme CuO.

$$0.296 \times 50 = 14.8.$$

$14.8 \times 0.4535 = 6.71$  per cent. reducing power expressed as dextrose.

The optical activity of fermented solution gave a reading of  $2^\circ$  in 200 mm. tube =  $10^\circ$  on sugar.

We have now data for working out our proportions of maltose and dextrose, thus :—

|  | Per cent.  |
|--|------------|
| Total sugar, expressed as dextrose ... ..      | 78.45      |
| Unfermented sugar expressed as dextrose ... .. | 6.71       |
|  | <hr/>      |
| Sugar removed by fermentation =                | 71.74      |
|  | <hr/>      |
| Total optical activity ... ..                  | $50^\circ$ |
| Optical activity after fermentation ... ..     | $10^\circ$ |
|  | <hr/>      |
| Optical activity removed by fermentation       | $40^\circ$ |
|  | <hr/>      |

The maltose and dextrose have both been removed by fermentation. The loss, therefore, in reducing and rotatory power is due to those substances, and from these combined figures we can calculate the proportion of each of these bodies in the mixture.

Now, maltose has a  $K^*$  61 and  $[\alpha]_D$   $135.9^\circ$ . Dextrose has a  $K$  100 and  $[\alpha]_D$   $51.7^\circ$ .

One part of dextrose therefore has an angle of  $0.517^\circ$ , and the reducing sugar (which has been calculated as dextrose), multiplied by this value, gives

$$71.74 \times 0.517^\circ = 37.1^\circ,$$

so that if our sugar were all dextrose, the angle corresponding with it would be  $37.1^\circ$ . The sugar has, however, an angle of  $40^\circ$ , or  $2.9^\circ$  above that required for true dextrose ( $40.0^\circ - 37.1^\circ = 2.9^\circ$ ), this excess angle being due to the presence of a proportion of maltose, which has a higher angle than dextrose.

In order to ascertain what proportion of maltose present in the sugars would cause a rise in the angle corresponding with that found, we must then proceed as follows:—

Maltose has an angle of  $135.9^\circ$ , but in our estimation we have expressed any maltose present in terms of dextrose, and therefore we must ascertain the angle which will correspond to maltose when thus expressed.

Now, the relative value of the  $K$  (or reducing power of the sugars, compared with dextrose) is dextrose  $K$  100, and maltose  $K$  61, and therefore as our maltose is expressed as dextrose, or  $K$  100 value, and not  $K$  61 (its true value), we must calculate the angle to correspond with this higher  $K$  value, thus:—

$$135.9^\circ \times \frac{100}{61} = 222.8^\circ [\alpha]_D.$$

\*  $K$  equals the reducing power of a sugar compared with dextrose, dextrose being taken as 100. 61 parts of maltose precipitate the same amount of cupric oxide as 100 parts of dextrose, therefore its reducing power is expressed as  $K$  61.

Having now ascertained this figure, we find that the difference between the angles of dextrose with K 100, and maltose expressed as of K 100 value, is  $171.1^\circ$

$$(M\ 222.8^\circ - D\ 51.7^\circ = 171.1^\circ).$$

In other words, a rise in the optical activity of  $171^\circ$  over the angle corresponding with dextrose would show that all the so-called dextrose was really maltose, and that the sugar in the sample contained 100 per cent. of maltose.

The rise in our sample is  $2.9^\circ$ . This will therefore equal

$$2.9 \times \frac{100}{171} = 1.7.$$

Therefore :—

|                         |     |     |     |     |       |
|-------------------------|-----|-----|-----|-----|-------|
| Total dextrose          | ... | ... | ... | ... | 71.74 |
| Dextrose due to maltose | .   | ... | ... | ... | 1.70  |
|                         |     |     |     |     | <hr/> |
| True dextrose in sample | ... | ... | ... | ... | 70.04 |
|                         |     |     |     |     | <hr/> |

The "dextrose" due to maltose has now to be dealt with, and the amount of maltose which it represents ascertained.

The "dextrose" being really maltose with K 100, is calculated into true maltose K 61 value, as follows :—

$$1.70 \times \frac{100}{61} = 2.79 \text{ per cent. maltose.}$$

The maltose in the sample, therefore, equals 2.79 per cent.\*

The residue after fermentation consists of gallisin, dextrin, and other unfermentable bodies, etc.

\* The above calculation is given at length, as the student frequently finds it difficult to work if only the formula is stated.

**Dextrin.**—The dextrin may be determined directly by precipitation if desired, and very good results have been obtained by the following method :—

The syrup is exhausted by repeated extraction with methylated spirit, using a glass rod for mixing syrup and solvent, and the precipitate of dextrin, which always contains some amount of maltose, however thoroughly the extraction has been carried out, is dissolved in hot water and made up to 100 c.c. The specific gravity, angle and copper reducing power are each determined on the solution in the way already described. The reducing sugars are calculated as maltose, and the specific rotatory angle due to this is calculated and subtracted from the total angle already found for the solution of the precipitate. The difference is then calculated as dextrin.

The percentage so found added to the maltose should give a total approximately agreeing with the dry solids obtained in the usual way by means of the factor 3.86 from the specific gravity of the solution.

*Example (Malto-dextrin syrup) :—*

The specific gravity of the dextrin precipitate dissolved up in the way described was 1.0131, and the total angle  $+56.3^{\circ}$ , which by determination of the reducing sugars was calculated to dextrin 16.74, and maltose 16.95, a total of 33.69.

The dry solids obtained by the factor  $3.86 = 33.94$ , the error in this case being therefore a very small one.

The estimation of gallisin is, for reasons described in an earlier section, very unsatisfactory, and it is sufficient for most purposes if, after deducting the protein, ash, etc., we group together the unfermentable matters, excepting dextrin if this has been estimated directly, and express them as such.

## NITROGEN.

Determine by Kjeldahl's method as before described.

*Example :—*

5 grammes sugar contained 0.025 protein = 0.50 per cent. on sugar.



## ASH.

Determine as in invert sugar.

*Example :—*

5 grammes gave 0.023 mineral matter = 0.46 per cent. on sugar.

## MOISTURE.

Determine as in invert sugars, making correction for ash as there described.

*Example :—*

The specific gravity of a 20 per cent. solution = 1067°.

$$67 \div 3.86 = 17.36$$

$17.36 \times 5 = 86.80$  per cent. of apparent solids in sugar.

The amount of ash was 0.46, and  $0.46 \times 2.07 = 0.95$ , and  $86.80 - 0.95 = 85.85$  solids due to organic matter. Adding to this the amount of mineral matter, we obtain  $85.85 + 0.46 = 86.31$ , total percentage of solid matter in sugar.

This deducted from 100 gives the moisture percentage, thus :—

$$100 - 86.31 = 13.69.$$

## UNFERMENTABLE BODIES.

Having determined the other constituents of the sugar, these bodies can be arrived at by deducting the sum of the dextrose, maltose, and albumen from the total organic solids, the difference being expressed as unfermentable matters.

*Example:—*

70·04 (dextrose) + 2·79 (maltose) + 0·50 (albumen) = 73·33,  
and 85·85 - 73·33 = 12·52 unfermentable matters.

## TESTS FOR IRON, COLOUR.

These are to be carried out as described under "Invert Sugar." Note carefully whether the solution given by the saccharine is brilliant, and boil some of the brilliant solution (filtering for this purpose if necessary) in a flask, for three or four minutes. Some saccharines throw a precipitate when thus treated.

The preceding results may be thus tabulated:—

|  |     |     |     |                 |
|--|-----|-----|-----|-----------------|
| Dextrose...                                      | ... | ... | ... | 70·04 per cent. |
| Maltose ...                                      | ... | ... | ... | 2·79 „ „        |
| Protein ...                                      | ... | ... | ... | 0·50 „ „        |
| Mineral matter ...                               | ... | ... | ... | 0·46 „ „        |
| Moisture ...                                     | ... | ... | ... | 13·69 „ „       |
| Dextrin, Gallisin, Unfermentable<br>bodies, etc. | ... | ... | ... | 12·52 „ „       |
|  |     |     |     | 100·00          |

These figures were obtained from an average starch sugar or "glucose," but fluid "malto-dextrins" are similarly analysed and contain more maltose.

There are a number of "special" sugars on the market, most of them being mixtures of cane syrups or the inversion products of cane sugar with one or other of the above mentioned starch sugars, and these special sugars may also contain caramel, liquorice, and other substances, such as a small percentage of the raw sugar residue washed away in the centrifugal machine previous to its refining. These sugars

are many of them suitable only for priming black beers, and their use is generally restricted to priming quick running beers for obtaining a particular fullness or lusciousness of flavour.

An accurate analysis of each component of such special sugars is by no means easy, but the method adopted must be based upon the different reducing powers and specific rotatory angles of the various sugars as described above, and the different conditions under which certain sugars may be hydrolysed; a direct precipitation of the dextrin as described above is often of added use for certain mixtures.

Suggestions have been made from time to time for analysing mixed sugars based upon differences in fermentability by certain pure culture yeasts. Such biological methods for sugar differentiation are, however, considerably beyond the capabilities of most brewery laboratories, and have not yet been formulated on a satisfactory basis.

### III.—CANE SUGARS.

These are analysed precisely as described under "Invert Sugars," but as they usually contain traces only of invert sugar, the determination of that substance is made on a 10 per cent. solution instead of a 1 per cent.

## INTERPRETATION OF SUGAR ANALYSIS.

### Invert Sugars.

Of these there are many qualities, varying with the price; but the standard No. 1 and 2 qualities of all manufacturers should attain a definite degree of purity and strength.

The following figures may be taken as fair requirements for the qualities named :—

|   | No. 1.                | No. 2.           |
|---|-----------------------|------------------|
| Invert sugar, not less than ... ..                  | 76.0 per cent. ...    | 74.0 per cent.   |
| Cane sugar, not more than ... ..                    | 1.5 per cent. ...     | 2.0 per cent.    |
| Mineral matter, not more than ...                   | 1.50 per cent. ...    | 2.0 per cent.    |
| So - called intermediate matters, not more than ... | 2.5 per cent. ...     | 3.0 per cent.    |
| Optical activity, not less than... ..               | - 10 $[\alpha]_D$ ... | - 7 $[\alpha]_D$ |

A large proportion of cane sugar may not in itself be objectionable, but it generally indicates either that a very low class sugar has been employed, or that crystals of cane sugar have been first separated, and the residual syrup has been used for inversion. The same remark applies to the presence of much mineral matter, or an excess amount of non-sugar carbohydrates. In any case such samples should be viewed with the gravest suspicion.

The angle of the sugar is very useful in determining the addition of glucose, which is sometimes added for the purpose of cheapening the cost of production, for improving the colour of the sugar, and for assisting it in solidifying. Brewers occasionally insist that their invert sugar shall be delivered in the fluid state. This is no doubt convenient in certain cases, but it must be pointed out that the better the sugar is, the more rapidly it becomes solid, so that by a too great insistence upon the point of fluidity, the brewer may encourage the manufacturer either to "boil light," in which

case he leaves more water in the sugar, and the extract is of course greatly lowered, or to leave some cane sugar uninvverted, as the presence of any considerable proportion of cane sugar retards solidification.

The proportion of unfermentable matter in an invert sugar is of importance, as showing the material from which it has been made. Probably it is not desirable that an invert sugar should be completely fermentable; it is likely that the unfermentable humous substances communicate considerable fulness, yet there should not be too great an excess of these.

The amount of iron in an invert sugar of No. 1 quality should not exceed a slight trace, that is to say, an amount, as quantitatively determined on p. 205, not greater than 0.005 per cent., expressed as oxide of iron,  $\text{Fe}_2\text{O}_3$ . For lower grade sugars, 0.025 per cent. is a heavy trace, and this amount of iron should be considered the maximum allowable, as it has been found, practically, that such sugars give unsatisfactory results when boiled with hops.

Invert sugars are now rarely prepared from beet, but if this has been used in their manufacture it may generally be detected by the smell, particularly noticeable on boiling the sugar solution.

Sulphites have been used to some extent as a bleaching agent in producing a pale sugar by adding this to the inverted syrup when concentrating in the vacuum pan, and if present are usually detected by the flavour. If suspected, a solution of the sugar may be distilled with a little dilute hydrochloric acid into bromine water, and estimated as described under "Sulphites."

Invert sugars should be not only sweet to the palate but of a full luscious flavour, and purity is essential for the production of beers required to keep sound for any length of time. These sugars are generally used in the copper up to 25 per cent., or even more in some cases, and for priming purposes by themselves or mixed with cane or other sugars.

Invert sugars are usually boiled down to give an extract of 35 to 36 lbs. per cwt.

### Starch Saccharines.

These vary so much in character that it is difficult to classify them. In practice it is found that those which contain the highest proportion of dextrose by no means give the best results. Dextrose or glucose is exceedingly fermentable, and during primary fermentation we may be certain that the whole of this will be split up into alcohol and  $\text{CO}_2$ ; yet if such saccharines consisted wholly of dextrose we could not expect that they would communicate any flavour or fulness to the beer, but merely serve as alcohol-producing material. But we know that the use of certain classes of glucose does undoubtedly give roundness of flavour; and whilst the value of a glucose, as well as of an invert sugar, may be partly due to its diluting influence upon the malt extract, yet this does not adequately explain the advantages known to be attendant upon their employment. It seems clear, therefore, that the presence of unfermentable matter is desirable in such materials. The amount of this will vary much, and, generally speaking, better results are obtained with samples containing a large, than with those containing but a small proportion.

The unfermentable matter consists of gallisin, dextrin, and other ill-understood bodies. Certain classes of sugars contain much dextrin, but this generally in association with considerable quantities of maltose also (as in "dextrin-maltose"), and undoubtedly such sugars do communicate a clean, dry fulness, which renders them very suitable for use in certain classes of beer, particularly in light-gravity bitter beers. In an ordinary glucose we may adopt the following general standards :—

The dextrose and maltose should amount to not less than 65 per cent. (in strong sugars—those containing a low moisture percentage—this figure should be higher). The dextrin will usually be about 1 to 2 per cent.; the proteins 0·5 to 1·5 per cent. A higher proportion than 2 per cent. is objectionable. The amount of ash varies from 1 to 1·7 per cent.

If the glucose has been prepared from starch, as distinct from whole grain, the proteins may be as low as 0·3 to 0·5 per cent., and the ash 0·3 to 0·6 per cent., but such samples give, as a rule, little or no fulness to the finished beer.

In the class of sugars known as dextrin-maltose, the acid conversion is arrested the moment the starch has disappeared, and, as a consequence, they contain much maltose and dextrin, and but little dextrose—in some cases practically none. This stage, however, must not be anticipated, or the sample may contain soluble starch, which is, of course, objectionable. It is therefore wise to test every such sample for starch with iodine. The proportions of maltose and dextrin present vary greatly.



The sugar is sent out as a clear and nearly colourless syrup, which, though viscid—owing to the large proportion of dextrin present in it—yet usually contains a high proportion of moisture, not infrequently as much as 22 to 24 per cent.

Such a sugar is, of course, only very partially and slowly fermentable, and is much used as priming, on account of the slow and prolonged “conditioning” which it induces, and that without rendering the beer sweet.

### Cane Sugars.

*Raw sugars* are, as a rule, only used for running mild beers (fourpenny) and black beers. There is no doubt that the peculiarly rich flavour which they communicate is of advantage in those cases where no great stability is required. For this purpose no special standard of purity need be laid down; a consideration of the particular requirements will determine the type and general quality to be employed. It is, however, certainly not advisable to employ the lowest class of such sugars, for they are almost invariably extremely impure.

A rough indication of the purity of the sugar is obtained by noticing the crystal formation, the larger and more completely these are formed the purer the sugar.

Raw or crude sugar invariably contains marked quantities of water, ash, invert sugar, and extractive matters. The ash of beet sugar contains less lime and phosphoric acid, but more potash than does that of cane sugar.

There is, however, a popular error regarding beet sugar, which requires correction. It is commonly supposed by

brewers to contain iron in considerable amount, and they justly fear the formation of the black tannate of iron in their coppers should the hops come in contact with a sugar solution containing iron. As a matter of fact it is singularly free from that metal, far more so than are many varieties of cane.

Low sugars, such as Jaggery, Egyptian, and Mauritius, contain notable amounts of invert sugar, as do also the syrups of the refiner after separation of the crystallisable portion of their constituents, the original percentage in this case being not only increased by the removal of crystallisable sugar, but very materially also by hydrolysis during the repeated boilings in pan which such syrup has undergone. Treacle and golden syrup will sometimes contain as much as 30 per cent. of invert sugar, while they will yield an ash as high as 4 to 6 per cent.

The presence of large quantities of mineral matter interferes with the crystallisation of cane sugar from its solution, and this causes much annoyance and loss to the refiner, preventing him from obtaining the whole of the cane sugar in crystalline form from his syrups. It is generally stated that one part of ash prevents the crystallisation of five parts of sugar from its solution, and indeed in its assay it is usual, before expressing the crystallisable sugar in any sample, to deduct from the total cane sugar found by analysis five times the weight of the ash, together with an equal weight of invert sugar. This rule is merely arbitrary, and is no doubt incorrect; the deterrent action of saline matter being actually about 3.75 times, and that of invert sugar twice its weight.

*Refined Sugars.*—Some of the higher forms of refined sugar now prepared are of remarkable purity, containing as much as 99·8 per cent. of absolute cane sugar with less than 0·1 per cent. of ash. Perhaps the purest form is that of the large perfectly colourless crystals, commonly known as “coffee” sugar, such being almost absolute cane sugar without any appreciable amount of ash or moisture.

In valuing refined sugars much depends upon the purpose for which they are to be employed. Considerable quantities are now used for inversion in the brewery, and for this purpose a rich-flavoured sample should be selected; but if the acid process is employed it must not be forgotten that considerable increase of colour occurs during inversion; whilst if the yeast inversion process is adopted no appreciable increase in colour occurs, but the inverted syrup is never so rich in flavour as with the acid-made sugar. If the acid process is adopted the sugar should not be of very low class, for the impurities, particularly the salts, prevent proper inversion.

A good method for inversion of sugar is as follows:—

A wooden vessel is required, which shall not have any interior metal fittings save one or two nozzles for admission of free steam; and these may be arranged so that the steam, by entering the liquid in a slanting direction, sets the whole of it in motion. For each cwt. of sugar to be inverted, 0·8 barrel of water (29 gallons) is placed in the vessel, and the sugar is then added, which will raise the total bulk to 1 barrel per cwt. (1 cwt. of sugar occupies, when dissolved, a space of about 7 gallons).

Raise the liquid to the boiling point. Then turn off steam and add 1 lb. of commercial sulphuric acid (oil of vitriol) per barrel of liquid. First dilute the acid with water in a wooden (not metal) pail, taking care to pour the acid into the water, not *vice versâ*. Now stir in the

diluted acid, turn on steam, and boil for one hour. If the sugar is of fair quality it will be inverted, but if of low character  $1\frac{1}{2}$  hours' boiling will probably be required. When boiled the correct time—and this must, in the first case, be ascertained by analysis—turn off steam, and slowly add a cream of whiting and water ( $1\frac{1}{2}$  lbs. whiting per 1 lb. of acid used). Add cautiously, with occasional stirring, as the solution is sure to froth, and does so specially with low-class sugars. Now again boil for two or three minutes, then turn off steam, and allow to settle. If the sugar is of good quality, the liquid will settle bright in about two hours, and may be run off. The deposit, which consists of a mixture of sulphate and carbonate of lime, may be washed with a little water, or strained through bags. One cwt. of good sugar will, after inversion, yield a saccharometer extract of 84 lbs.

Tompson's yeast inversion process consists of the addition of yeast to a solution of the sugar, at a temperature of about  $130^{\circ}$  F., when the maximum invertive power of the yeast is exerted, but no fermentation is possible. After some hours the liquid is boiled, the yeast skimmed off (not all can be thus removed), and the liquid, which is somewhat opalescent, run direct into the copper. The invert sugar thus produced is more completely fermentable, but less rich in flavour, than that produced by acid.

When cane sugar is used for priming, it is necessary to insist on a higher state of purity. The candy sugar frequently used for this purpose contains over 99 per cent. of actual cane sugar, and mere traces of impurities. It has often been stated that such sugar, if made from beet, is liable to produce ropiness, but there seems to be no proof of this, and in view of the extreme purity of such sugar (even when prepared from beet) it hardly seems probable that this can be the case. It is, of course, most important that the solution of sugar shall be well boiled, and prepared in small quantities at a time. The practice of holding over such syrups—often in an unheaded cask—for several days is much to be deprecated.

## Composition of Raw Sugars and Syrups.

|                              | Raw<br>Jaggery. | Raw<br>Penang. | Raw<br>Egyptian. |
|------------------------------|-----------------|----------------|------------------|
| Cane Sugar ... ..            | 76·20           | 77·10          | 81·00            |
| Invert Sugar... ..           | 10·50           | 10·20          | 3·90             |
| Other Organic Matters ... .. | 3·20            | 2·40           | 0·70             |
| Mineral Matters ... ..       | 5·20            | 3·20           | 8·30             |
| Moisture ... ..              | 4·90            | 7·10           | 6·10             |
|                              | 100·00          | 100·00         | 100·00           |

|                 | "Green<br>Syrup." | Treacle. | Molasses. | Refined<br>"Moist." | American<br>Cane<br>Syrup. |
|-----------------|-------------------|----------|-----------|---------------------|----------------------------|
| Invert Sugar    | 15·43             | 26·30    | 18·00     | 6·54                | 31·79                      |
| Cane Sugar...   | 50·40             | 34·39    | 48·00     | 85·24               | 34·98                      |
| Albuminoids     | 2·38              | 2·53     | ...       | 0·65                | 0·81                       |
| Ash ... ..      | 3·67              | 4·91     | 1·40      | 1·74                | 6·50                       |
| Moisture ... .. | 16·91             | 17·06    | 31·10     | 3·57                | 23·55                      |
| Other Bodies    | 11·21             | 14·81    | 1·50      | 2·26                | 2·37                       |
|                 | 100·00            | 100·00   | 100·00    | 100·00              | 100·00                     |

## CHAPTER VI.

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**CARAMEL.**

THE following determinations should be made :—

- Colouring power.
- Action upon water.
- Action upon bright beer.

In addition to these, in certain cases, it is desirable to add the following tests :—

- Fermentability.
- Cupric oxide reducing power.
- Optical activity.
- Nitrogen.

**COLOURING POWER AND ACTION UPON WATER.**

Make up a 10 per cent. solution of the caramel by weighing out 10 grammes, dissolving in distilled water and diluting to 100 c.c. at 60°. Now measure 10 c.c. of this solution into a litre flask, make up to the mark, and thoroughly mix = 0·1 per cent. solution.

Note whether the whole of the caramel dissolves and whether the solution is brilliant or cloudy. If not brilliant filter a small quantity through a dry paper before estimating the colour.

Determine this by means of the Lovibond tintometer, reading the solution in a 1-inch cell and matching the colour by means of the 52 series of glasses. The result is expressed as the colour in a 1-inch cell 52 series glasses 0.1 per cent. solution.

This is the method of expression for the colour of caramel, as advised by the Caramel Committee appointed by the Institute of Brewing, 1910, but, if required, the colour can readily be calculated to a percentage on the caramel.

## ACTION UPON BRIGHT BEER.

Dissolve a little of the caramel in some bright beer, and with this colour some more of the same bright beer to a deep brown tint. This should be placed in a corked or stoppered bottle. Set it on one side, and examine after about 24 or 48 hours. There should then be no appreciable amount of deposit.

The bright beer used for testing the caramel in this way should itself be kept under the same conditions for comparing with the caramel-treated beer, as newly fined green beer will often throw some amount of deposit, or become hazy a short time after bottling. The caramel should also be tested with beers of both heavy and light gravity.

Some caramels when exposed to strong light in water or beer solution are found to lose a considerable percentage of colour density after standing for some days, and as much as 10 per cent. loss in colour has been observed after a week, no precipitation having taken place in the meantime.



## FERMENTABILITY.

The fermentation experiment is conducted by diluting the caramel to a gravity of about 1050, pitching with yeast, placing on forcing tray, and noting the loss in gravity after fermentation. Before taking the gravity after fermentation, any alcohol present must be removed by boiling, the solution being then cooled, made up to the original bulk, and filtered bright.

A fermentation should also be carried out with a mixture of caramel and wort, and the colour observed before and after fermentation.

Some caramels will lose as much as 50 per cent. of colour in this way.

*Example :—*

Two caramels of high colouring power gave results as follows :—

|                                     | 1.     | 2.     |
|-------------------------------------|--------|--------|
| Colour expressed on the caramel ... | 42,000 | 48,000 |

These were fermented, using 10 grammes of caramel, 200 c.c. of 1050 gravity hopped wort, with  $\frac{1}{2}$  gramme of yeast. After fermentation had finished the colours were again taken and following results obtained :—

|                                     |            |        |
|-------------------------------------|------------|--------|
| Colour after fermentation expressed |            |        |
| as per cent. on caramel ...         | ... 30,800 | 35,200 |

These expressed as percentage on the original caramel show a loss during fermentation of 26·6 per cent. in each case.

## REDUCING POWER AND OPTICAL ACTIVITY.

It is sometimes customary to determine the copper oxide reducing power and specific rotatory power of a caramel, and the determination of the former may be made as in the case of invert sugar, but as the reducing power of a caramel does not average more than 30 to 40 per cent. expressed as dextrose, a 2 per cent. solution of the caramel must be used for the gravimetric process in place of 1 per cent. as used for sugar. The fact that most caramels have some reducing power does not by any means indicate that they will be fermentable to the extent of the reducing sugars shown, for as a rule caramels are very little fermentable.

With regard to the specific rotatory angle, it has been suggested that good caramels have generally a low optical activity, bad ones usually a high opticity, but it is doubtful if any very great value can be attached to such figures. To determine the angle the caramel must be decolorised as described under "Beer Analysis."

## NITROGEN.

Caramels are now very often made from glucose instead of cane sugar, and under these conditions it is found that a caramel may show a considerable percentage of nitrogen. The total nitrogen on a caramel can be determined by the Kjeldahl method exactly as in the case of invert sugar, and the percentage of nitrogen expressed as protein may be found to be as high as 10, or even 15 per cent. Nitrogen

in caramel may also be due to use of ammonium salts in the manufacture, and in this case the ammonia is detected and estimated by treating a known quantity of caramel dissolved in water with soda solution and distilling into standard N/10 acid, as described for the determination of nitrogen in malt.\*

The presence of any considerable percentage of nitrogen may to some extent lower the stability of the caramel, although some experiments we have made indicate that such nitrogen is not assimilable by yeast during normal fermentation, and is presumably not a food supply for such organisms as yeast or bacteria. A similar remark holds good for the nitrogen when present as ammonium compounds.

It is not precisely known how far such nitrogenous bodies are harmful in brewing operations, and much depends upon the particular class of beer which is to be brewed, and whether the caramel is to be used as a percentage on the grist, in the copper or as a priming, but, generally speaking, it would seem not advisable to use a highly nitrogenous caramel for brewing a beer expected to keep for any length of time, such as the heavier gravity stouts, and certainly not those intended for export purposes. For use in copper for running beers such as porter or as a priming, such nitrogenous caramels are probably not harmful.

\* A portion of the nitrogen in the ammonia appears to enter into combination with certain constituents of the caramel.

## CARMEL PREPARATION AND INTER- PRETATION OF ANALYSIS.

### Carmel.

The term carmel is generally applied to the liquid or solid substances very commonly used in brewing for colouring and flavouring purposes. It is prepared either from starch saccharine (commercial glucose) or from cane sugar. It may also, but with difficulty, be prepared from starch. The material does not appear to differ much, from whichever of the above substances it is prepared. It is made\* by heating cane sugar (raw sugar is occasionally used) or glucose to a high temperature. The sugar at first melts, and as the temperature rises, water is given off. This continues until a temperature is reached at which violent action commences, and irritating fumes and much steam are evolved. The process is allowed to continue until the maximum colour has been developed, the temperature rising to from 370° to 400° F. Water is then run in, and the solution thus formed is filtered and evaporated. The process is in practice extremely difficult to carry out successfully. If the heating is not carried sufficiently far, the tintorial power of the carmel will not be deep enough, yet if carried very little beyond the necessary point (and the temperature is

\* See also a paper by A. Gordon Salamon and E. M. Goldie, "The Manufacture of Carmel," *Journ. Soc. Chem. Ind.*, 1900, pp. 301-307.

not easy to control), the whole will be converted into a mass of carbon. The true colouring matter of caramel is said to have the formula  $C_{125}H_{188}O_{80}$ , and is thus formed :—



obtained by precipitation from ordinary caramel with absolute alcohol, and by subsequent dialysis. It is clear, however, that ordinary commercial caramel does not consist merely, or, indeed, chiefly, of this body. Evans states that he has found ordinary caramel to contain about 30 per cent. of the dry solids of true colouring matter, and 70 per cent. of an inert body, precipitable by sulphuric ether, and drying as a white, very deliquescent mass, which is readily soluble in water, but absolutely unfermentable. The above statement of the perfect unfermentability of commercial caramels cannot be corroborated in the writers' experience. It is a matter on which there is some difference of opinion whether a caramel from a glucose or a cane sugar is the better. On the whole, probably the latter gives a richer flavoured product. In order to intensify the colour about 0·5 per cent. of sodic or ammoniac carbonate is sometimes added, either during or after the heating process. As a consequence of the great difficulty that exists in making a satisfactory caramel, commercial samples are most variable in quality. Many caramels are not perfectly soluble in water. In solid caramels there is almost invariably a residue of insoluble carbon, but even if soluble they not infrequently throw down a heavy precipitate when added to beer. This precipitate contains nitrogen, and appears to be some compound of caramel with protein.

With some beers, the precipitate is much more marked than with others.

Caramel is of course used for the purpose of adding colour to the wort, but the *flavour which it communicates is of very great importance*. This varies considerably. Some caramels possess a rough, harsh flavour, often found decidedly objectionable, although those that are richest in flavour are not, as a rule, very high in colouring power. The tintorial power of caramels varies within wide limits—some are as high as 48,000°, and a good liquid caramel should certainly have a colouring power of not less than 25,000° to 30,000°. As already mentioned, the colouring power is not always permanent, but is frequently precipitated when added to beer; and thus not only is the available colouring power much lower than that apparent, but, what is perhaps most serious, a cloud is produced in the beer treated. No caramel can be passed as satisfactory which throws a cloud when added to bright beer. In many cases when caramel is added to the copper there is a considerable loss of colour during fermentation. There is normally, of course, some reduction in the colour of a wort when fermented with yeast, but this is not considerable, and is quite distinct from the loss of colour which occurs with some caramels. Most caramels are partially fermentable, but considerable fermentability indicates an imperfectly prepared sample, which will probably be of low tintorial value, for it shows the presence of undecomposed sugars; whereas, in a well-prepared sample, the greater proportion of the carbohydrate bodies in the original sugar should have been converted into unfermentable matters.

The presence of insoluble carbon in solid caramels necessarily reduces their tintorial and extract values, but, beyond this, the presence of carbon does not of itself prove the sample to be a bad one. Both the colour and flavour of a caramel are very different from that imparted by black, or so-called "patent," malt ; 1 cwt. of good caramel may be taken as roughly equivalent in colouring power to  $1\frac{1}{2}$  cwt. of good black malt.

In some special caramel preparations liquorice is used, and no doubt a certain richness of flavour is obtainable from the employment of a small proportion of this substance. The flavour of liquorice is, however, so penetrating that only very small quantities should be employed.

Some caramels are left very acid after manufacture, whilst others are neutralised or actually made alkaline. The flavour is certainly very different in the three cases, and as a rule it is not desirable to use a caramel with a high acidity. If, on the other hand, it is over neutralised, it will have a peculiar unpleasant smell and flavour. Caramel should show only a slight trace of acidity.

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## CHAPTER VII.

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**H O P S .**

IN the analysis of hops the following determinations should be made :—

1. Foreign matter.
2. Moisture.
3. Soft and hard resins.

These must be supplemented by a careful physical examination. The hops should also be tested for sulphur, and a method is given for the determination of tannin.

**FOREIGN MATTER.**

It is most important to obtain a satisfactory sample of the hops under examination, and a flake should be taken for analytical purposes as representative of the pocket as possible. Samples are sometimes found to include sweepings from the hop floor, which are, as is well known, added from time to time to the pocket. Such a sample must be rejected, for it does not fairly represent the contents of the pocket, although, notwithstanding its dirty appearance, it will be found to contain a high percentage of resins, as dust from the floor consists chiefly of the resinous powder which has been separated from the hops during manipulation.

The sample taken will be found to include leaves, pieces of stem, and other extraneous matter, such as, in some cases, pieces of earth and clay.

One or two separate samples of, say, 50 grammes weight should be taken, the foreign matter carefully separated, and the average percentage found.

This determination of foreign matter is the more important, inasmuch as the resin value of the hop is determined on the complete hop flower, so that the extraneous matter in the sample must be taken in conjunction with the resin value when considering the general brewing quality of the hop as a whole.

### MOISTURE.

This is determined by drying 3 to 5 grammes of the hops in the boiling water oven for five hours. Some, if not all, of the oil is driven off by this process, but as it does not exceed 0.5 per cent., the error thus involved is not very great. If necessary, a correction may be made for this.

The method of drying over sulphuric acid *in vacuo* is preferable, but the above process yields results sufficiently reliable for technical purposes.

### SOFT RESINS.

A number of complete or whole flowers are separated from the sample of hops under examination, and any stalk which may be attached is carefully broken off close to the flower and discarded.

Five grammes of the flowers so obtained are weighed out, placed on a piece of glazed paper or glass, each flower care-

fully broken open by means of forceps, and the whole transferred to a dry graduated 515 c.c. flask of somewhat wide neck, similar to that used for the determination of extract in malt.

Five grammes of hops are calculated to occupy a volume of about 2.5 c.c.,\* so that the flask must be re-marked for this capacity, or a correction afterwards made if the volume is finally made up to the 515 c.c. mark.

Having carefully brushed all the hops into the flask, 400 c.c. of petroleum ether are then added, and kept gently boiling in a water bath under a reflux condenser for eight hours.

The extraction should include, if possible, at least one night's soaking. At the end of this time, cool the flask contents down to 60°, and make up to the mark with petroleum ether. Thoroughly mix and filter through a dry paper and funnel into a dry stoppered bottle, taking care not to lose any of the hop matter afterwards to be used for the determination of the hard resins.

When a sufficient quantity of the petroleum ether extract has filtered through into the bottle the funnel is withdrawn, and 100 c.c. of the extract measured out into a small wide-mouthed flask of known weight, and the ether gently evaporated in a water bath at 120° by distillation through a small Liebig condenser. When the great bulk of the ether has been removed in this way, the flask is allowed to stand on the top of the boiling water bath for a short time and afterward dried inside the water oven, its weight being taken

\* A. C. Chapman calculates the volume occupied by 10 grammes of hops as 8 c.c.—*Journ. Inst. Brew.*, 1907, p. 649.

after four hours, and the drying continued until the weight remains constant.

The changes that take place in the soft resins when dried by exposure to air are not yet well known, but it is probable that some oxidation results, and it would no doubt be safer to dry the resin in an atmosphere of hydrogen or coal gas by passing a slow current of the gas through the flask contained in the water oven.

Great care must be exercised when using petroleum ether, as it is highly inflammable. It is a product obtained from petroleum, and consists of the lower members of the paraffin or marsh-gas series. It contains pentane and hexane, with their respective isomerides. Pentane has a boiling point of about  $100^{\circ}$  F., and hexane one of about  $125^{\circ}$  F., while the isomerides boil at some  $10^{\circ}$  lower. This explains the want of uniformity in the boiling point of petroleum ether, but it should, as far as possible, have a boiling point of about  $120^{\circ}$ .

The extractive value of petroleum ether for the soft resins of hops is found to vary considerably with its boiling point, so that this should be obtained as uniform as possible, particularly where a comparative series of soft resins are determined in a number of hops.

In the last edition of this book, the method for hop extraction was somewhat different, the process being carried out in a Soxhlet's extractor, as in the determination of oil described on p. 164. Extracting by boiling in a flask as above described was found, however, after a number of comparative experiments, to be much more expeditious, and to give quite as accurate results.

**Lintner's Method.\***—The method of extracting in flask is due to Lintner, who, however, worked somewhat differently, using 10 grams of hops, and estimating the soft resins by adding 80 c.c. of absolute

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\* *Zeitsch. f. d. ges. Brau.*, 1898, p. 407.

alcohol to 100 c.c. of the petroleum ether extract, and titrating this solution with N/10 alcoholic potash, using 10 drops of 1 per cent. phenolphthalein solution as indicator. One molecule of alkali neutralises one molecule of lupulinic acid (molecular weight 400), and the amount of alkali used multiplied by 0.4 gives the weight of acid present.

Lintner had already found that the soft resins were acid in character, and accordingly devised the above method for their estimation. There are, however, a good many difficulties in the process, and as a result of a number of experiments made in our laboratory, working with both methods comparatively, we concluded that the best results were obtained by actually weighing the dried resin as above described.

Lintner describes a still more recent method for estimating the resins, in the *Chem. Zeitung*, 1908.\* After extracting 10 grammes of the crushed hops with ethylic (sulphuric) ether in a Soxhlet apparatus for 8—10 hours, the ether is evaporated, first on a water bath, and finally *in vacuo*, and the residue dissolved in methyl alcohol, the insoluble wax filtered off, and the filtrate made up to 100 c.c. Ten cubic centimetres of this solution are now treated with a 1 per cent. solution of lead acetate in methyl alcohol, avoiding excess of this reagent by testing a drop of the resin solution on a doubled piece of porous paper, when an excess of the lead solution will be indicated if the lower sheet is coloured brown by sodium sulphide solution. The precipitate is collected in a Gooch crucible, washed with methyl alcohol and ether, dried, and weighed. This precipitate is the lead compound of the  $\alpha$ -resin or humulone and has a lead-content of 36.69 per cent., so that the  $\alpha$ -resin can be easily calculated.

The sum of the resins known as  $\alpha$ ,  $\beta$ , and  $\gamma$  is next determined by evaporating 10 c.c. of the previously obtained methyl alcohol solution and weighing the dried residue.

Finally the sum of the  $\alpha$ - and  $\beta$ -resins is obtained by extracting the hops in petroleum ether. In this way each of the constituents,  $\alpha$ -,  $\beta$ -, and  $\gamma$ -resin, can be separately estimated.

## HARD RESINS.

The hops after filtration of the soft resin extract are now washed two or three times with a little petroleum ether,

\* See also *Journ. Inst. Brew.*, 1909, p. 578.

rinsing the hops in the flask, and also any that may have been transferred to the filter paper, such washings being collected in a separate stock bottle. Any hop matter left in the filter paper is now carefully transferred back again into the flask, and about 400 c.c. of sulphuric ether added, extraction being again made exactly as described for the soft resin. In this case, however, it is unnecessary to extract for more than four hours. The flask is then cooled back and made up to the mark at 60° with sulphuric ether, thoroughly shaken, and the liquid filtered through a dry filter paper into a dry bottle; 100 c.c. of this filtrate are evaporated and dried in the water bath exactly as in the case of the soft resins. The hard resin will be found to dry much more easily than the soft, and a constant weight should be obtained within a few hours.

## TANNIN.

A method for the estimation of tannin has recently been devised by A. C. Chapman, and is fully described by him in two papers read before the Institute of Brewing.\*

The method is much superior to that of Lowenthal, and is based upon the precipitation of tannin from an aqueous extract of hops by a solution of cinchonine sulphate, and is as follows :—

Ten grammes of the hops are extracted in a flask marked at 508 c.c. with 400 c.c. of boiling distilled water. The flask and contents are then immersed in a water bath, and kept gently boiling for two hours, then cooled, and made

\* *Journ. Inst. Brew.*, 1907, p. 646, and 1909, p. 360.



up to the mark with cold distilled water (508 c.c. are taken since by experiment the average volume occupied by 10 grammes of hops is found to be 8 c.c.). The hop extract is then filtered through a dry filter paper and 50 c.c. of the filtrate evaporated in a small beaker to about 15 c.c. on a boiling water bath, cooled, and 50 c.c. of a saturated aqueous solution of cinchonine sulphate added.

The precipitated solution should stand for an hour or two in a cool place, after which it is filtered through a weighed porcelain Gooch crucible containing a bed of prepared asbestos, which latter must first be rinsed with 0.5 per cent. cinchonine sulphate (one volume of the saturated solution diluted with one volume of water) and afterwards dried to a constant weight before filtering the precipitated hop extract through it. This latter is decanted into the crucible and allowed to filter at first without the aid of a pump, and when about half the liquor has run through suction should be applied and the whole of the precipitate transferred to the crucible. When all the liquor has filtered through, the precipitate is washed several times with 0.5 per cent. cinchonine sulphate, the same solution being used for washing out the beaker, care being taken that the precipitate does not dry on the sides. The washing is completed and the suction continued until the precipitate is moderately dry, shown by its tendency to break into pieces. The crucible and contents are then dried in a boiling water oven and weighed at intervals until constant. The weight of the precipitate so obtained multiplied by 0.55 gives the amount of tannin in 1 gramme of hops, from which the percentage is easily obtained.



## SULPHUR.

The presence of sulphur may be easily determined in either of two ways. In the first, we pass a current of hydrogen through a decoction of hops, when, if present, the sulphur will unite with that gas to form sulphuretted hydrogen ( $\text{SH}_2$ ), which may be detected by its characteristic odour of rotten eggs. Secondly, by passing the gas into a solution of plumbic acetate, when a brown or black precipitate or coloration is produced from formation of sulphide of lead ; or by suspending over the gas a paper dipped in such solution, which will then, in the presence of sulphuretted hydrogen, turn brown. The hydrogen for this purpose is generated from zinc and dilute sulphuric acid in a small flask, to which is fitted a cork with a bent tube passing through it, to conduct the gas into the hop solution. If the estimation is to be quantitative, the flask containing this solution must also be fitted with a cork furnished with two holes, through one of which, and descending almost to bottom of flask—so that the gas bubbles through the liquid—is the tube connected with the hydrogen-generating flask ; while through the other hole is a tube descending inside the flask only about half an inch, and bent externally in such a manner that the gas passes into a small flask or beaker containing a solution of plumbic acetate. The precipitated sulphide of lead is filtered, washed, dried, ignited, and weighed, the sulphur being calculated from the weight so found. One part of  $\text{PbS} = 0.1345$  sulphur.

## PHYSICAL EXAMINATION AND INTERPRETATIONS.

The physical examination of hops is of the utmost importance, for though analysis may give us information of the total preservative value of a hop, it is altogether unable to inform us of the flavour, aroma, etc. Unfortunately, written directions on such a matter are of small value, for long experience is necessary to form a sound judgment. The following points may, however, be mentioned :—

*Brightness.*—A good sample should be bright in colour, a dull, rusty appearance being generally an indication of a damaged hop.

*Condition.*—Examine the sample at the sides where the sampling knife has cut through the hops. Here the brewing value may be judged by the thick or thin appearance of the sample. In a rich sample the sections of the cones are seen laden with rich yellow condition, which, on rubbing down in the hand, is soft and oily.

*Management.*—A good insight into the management of hops during drying and packing is obtainable from the value of the sample when pressed downwards by the hand. An elastic springing sample indicates good management on the kiln and cooling floor, whilst a hard, non-elastic sample shows the reverse.

*Flavour.*—The delicate differences in the flavour of hops are not describable in words, but a practised observer is well able to ascertain if a sample has been properly cured, fired, or undercured. A “fired” sample lacks the true aroma

of a new hop, whilst there is present at the same time a slight flavour of fire. Such a hop will keep well, but is in other ways unsatisfactory. Fired hops, however, often lose some of the fire flavour when they have been kept for some time. An undercured sample has a distinct rawness of smell and aroma, and the sample lacks elasticity. If the strigs adhere to the cone, and bend rather than break, this is an indication of undercuring. A "cold" sample of hops is one which has absorbed too much moisture on the kiln floor. The determination of the moisture percentage is useful in this connection.

*Ripeness.*—A good hop should be ripe. Many of the very pale-coloured hops are picked too early, and being unripe do not keep well, and are also low in preservative value, for the amount of the preservative resins rapidly increases during the last stages of ripening. A brown-coloured hop is not necessarily objectionable, nor will it give increased colour in the copper if the brownness is merely due to ripeness. If, on the other hand, it is due to disease, there will be a considerable increase of colour. Haberlandt has proposed the sampling of hops by separating the "condition," sifting, and weighing it. This process is, however, unsatisfactory, and the total resins may be far more satisfactorily ascertained in the manner before described.

*Moisture.*—The percentage of moisture in the hop is of considerable importance, more particularly if the hop is intended for storage for any length of time. The amount should not exceed 10 per cent., and if much more than this is present the hop will not keep as well—8 to 9 per cent. is even better.

During drying of the green hops the moisture is generally reduced to from 3 to 6 per cent., but rapidly increases to from 7 to 12 per cent. from subsequent exposure on the packing floor.

*Resins.*—The yellow lupulin of the hop is admittedly the most valuable part of the hop plant to the brewer, and these “glands” contain not only the resins but also essential oil, wax, and other substances.

The resins have been separated into three well-defined substances:—The  $\alpha$ -resin, or humulone; the  $\beta$ -resin, or lupulinic acid, which two resins comprise the “soft” resins extracted by petroleum ether, and are both bitter and possess antiseptic properties; the  $\gamma$ -resin, which is tasteless and supposed to have no preservative value. During the process of copper boiling not much more than 50 per cent. of these resins are taken up by the wort.

*Essential Oil and Aroma.*—The small percentage of essential oil, 0.2 to 0.5 per cent., is also lost during the boiling stage of the brewing process, volatilising with the steam, but the aroma possessed by the essential oil no doubt, to some extent, affects the less volatile constituents of the hop and so influences the finished beer. The aroma is not so lost in dry hopping, and the hop for such purpose should be carefully examined for “mustiness,” from damp packing, and other unsatisfactory properties of a dry hop. It was at one time thought that the well known cheesy odour developed in old hops was due to oxidation of the essential oil to valeric acid, but A. C. Chapman has shown that this substance is never formed by aerial oxidation of the hop oil. It is probably produced from the resin or bitter of the hop.

*Mould*, as a whole, first appears on the leaf of the growing hop, and may afterwards develop on the flower, particularly if the vitality of this latter is checked during the ripening stage by cold or wet weather.

*Sulphur* is used both for sprinkling the growing vine to prevent mould, and is also thrown on the fires over which they are dried, when sulphurous acid, not sulphur, is introduced. This practice, although viewed with grave disapprobation by many brewers, has hardly been proved to be an injurious one, except in so far as its use disguises to some extent the real character of the hops, for a sulphured hop generally keeps better than an unsulphured one, and certainly is of a brighter and more attractive colour. Sulphur treatment in kilning is rarely practised abroad, but is almost universal in this country.

It is said that, when sulphur is sprinkled after the hop-flower has developed, it produces a peculiar smell in the dried hop, much objected to by brewers, and it is certain that yeast difficulties have with good reason been attributed by brewers to the use of heavily sulphured hops.

It is most important also not to mistake the bright and more or less green appearance of an unripe hop for a sulphured one.

*Tannin*.—It was originally thought that the tannin of the hop was of value in precipitating the proteins of wort, and that this percentage decreased as the hop became older. Recent experiments by A. C. Chapman, using his cinchonine method of estimation, have, however, finally disposed of this latter theory, and he has shown that the tannin percentage cannot be considered to give any valuable

indication of the brewing quality of the hop, thus confirming the statement made by Briant and Meacham in 1897, that "tannin certainly cannot be regarded as an index of the value of hops, either with regard to preservative power or flavour."<sup>\*</sup>

As regards the precipitating value of tannin, it has been shown that the nitrogen removed from a wort on boiling with hops belongs to the class not assimilable by yeast, and that the nitrogen directly contributed by the hops to the wort is, on the other hand, of the assimilable type, and, in fact, the effect of boiling a wort with hops is to slightly increase the assimilable nitrogen.

*Seeds.*—The seeds or fruit of a hop increase the weight of it without effecting any improvement in the brewing value, and these should therefore be as few as possible in number. In a ripe hop they are dark purple in colour; in an unripe one, greenish, and often shrivelled in appearance.

The percentage found is very variable, and may be from 10 to 25 per cent. They also differ considerably in weight

An excellent monograph on the hop and its constituents, edited by A. C. Chapman, 1905, and a book dealing more with the growth and management of the hop, by E. Cross, should be read.

### *Resin Composition of Hops.*<sup>†</sup>

The following figures are expressed on whole hops, and are the average of a number of samples :—

<sup>\*</sup> Briant and Meacham, *Journ. Fed. Inst. Brew.*, 1897, p. 82.

<sup>†</sup> See also a recent paper by Adrian Brown and G. Ward "On the Valuation of the Antiseptic Properties of Hops," *Journ. Inst. Brew.*, p. 641, 1910.



| New Hops.         |     |     | Hard Resins. | Soft Resins. | Total Resins. |
|-------------------|-----|-----|--------------|--------------|---------------|
|                   |     |     | Per cent.    | Per cent.    | Per cent.     |
| East Kent         | ... | ... | 3·91         | 10·65        | 14·56         |
| Sussex            | ... | ... | 5·30         | 9·12         | 14·42         |
| Worcester         | ... | ... | 5·12         | 7·60         | 12·72         |
| Goldings          | ... | ..  | 4·25         | 11·23        | 15·48         |
| Californian       | ... | ... | 8·45         | 12·20        | 20·65         |
| Bavarian          | ... | ... | 8·20         | 11·30        | 19·50         |
| British Columbian | ... | ... | 8·55         | 12·30        | 20·85         |
| Hallertauer       | ... | ... | 7·60         | 11·90        | 19·50         |

*Alteration by Age.*

|             |     |     | Kent.        |              | Californian. |              |
|-------------|-----|-----|--------------|--------------|--------------|--------------|
|             |     |     | Hard Resins. | Soft Resins. | Hard Resins. | Soft Resins. |
|             |     |     | Per cent.    | Per cent.    | Per cent.    | Per cent.    |
| New         | ... | ... | 3·80         | 10·20        | 8·40         | 12·80        |
| Yearlings   | ... | ... | 7·10         | 6·80         | 10·10        | 10·25        |
| Three Years | ... | ... | 9·50         | 3·90         | 13·20        | 5·23         |
| Five Years  | ... | ... | 12·50        | 1·60         | —            | —            |

It will be noticed that the deterioration is more rapid with the English than with the foreign hops.

The rate of deterioration depends on temperature and moisture, mainly the former. At temperatures of 45° F. and under, hops may be preserved almost unchanged for several years.



The following table\* gives figures showing the influence of temperature on the amount of soft resin present, expressed on the dry hop —

|                                      | Soft<br>Resins. | Hard<br>Resins. | Total<br>Resins. |
|--------------------------------------|-----------------|-----------------|------------------|
|                                      | Per cent.       | Per cent.       | Per cent.        |
| Hops as put in bottle ... ..         | 11·7            | 3·1             | 14·8             |
| Hops stored 7 months at 72—75° F.... | 8·8             | 5·9             | 14·7             |
| "      "      55—65° F....           | 9·2             | 5·1             | 14·3             |
| "      "      35—40° F....           | 10·6            | 4·2             | 14·8             |
| "      "      below 32° F....        | 11·1            | 3·5             | 14·6             |

\* "Hops," Briant and Meacham, *Journ. Inst. Brew.*, 1905, p. 14.

## CHAPTER VIII.

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WATER ANALYSIS.

It is first necessary to make a general examination, after which the following determinations are to be made :—

*Organic Matter.*

1. Ammonia saline, or “free.”
2. Ammonia “albuminoid.”
3. Oxygen absorbed.

*Inorganic Matter.*

4. Sulphated saline residue.
5. Lime.
6. Magnesia.
7. Alkalies (soda and potash).
8. Sulphuric acid.
9. Nitric acid.
10. Nitrous acid.
11. Chlorine.
12. Alkalinity before and after boiling.
13. Lead and other heavy metals.
14. Iron.

Determinations sometimes necessary :—

15. Silica.
16. Carbonate of soda.

## GENERAL EXAMINATION.\*

On opening sample, notice whether it is clear or not. If cloudy, add a drop or two of hydrochloric acid to a small quantity, and warm slightly, when the cloud, if due to carbonate of lime, will disappear. Place some of the water in a tall glass cylinder, and observe whether it has any colour. For this purpose the cylinder should be placed on a white porcelain slab, or sheet of paper, and examined, looking down through the column of the liquid.

Place some (about 200 c.c.) in a stoppered bottle, and allow to stand for a day or two—the analysis is, of course, proceeded with meanwhile—then pour off the water, and examine the sediment, if any, under the microscope. Observed whether deposit is crystalline, amorphous, or organised.

In making chemical analysis the determination of ammonia and oxygen absorbed should be made as soon as possible after the bottle or jar is opened. The organic analysis is therefore described first.

## ORGANIC MATTER.

### 1 & 2. Ammonia, Free and Albuminoid.

This is estimated by Wanklyn's process, in which the ammonia present in a water in its natural state is found by distillation and determination of the ammonia in the distillate by means of a most delicate test by

\* But if it is desired to make a *bacteriological examination* this must be done immediately on opening the bottle, and the water itself should have been specially collected in sterile bottles. For description of methods of analysis, see p. 396.

Nessler.\* By its means one part of ammonia in 10,000,000 parts of water may be clearly detected. The principle of the Wanklyn method is the measurement of the nitrogenous organic matters in water by the quantity of ammonia yielded by their destruction.

The agents used for the destruction of the organic matter are permanganate of potash, and an excess of caustic potash. In the interpretation of the results of analyses by this method, it must be borne in mind that but few organic substances give up the whole of their nitrogen as ammonia, and further that almost every substance yields a different proportion. Wanklyn says that "the disintegrating animal refuse would be pretty fairly measured by ten times the albuminoid ammonia which it yields"—a statement which must be accepted with considerable reservation.

To conduct the experiment—

Carefully clean a distillation flask of the kind used for the determination of original gravity in beer, and rinse through the spiral condenser with a little tap water. An ordinary retort attached to a Liebig still may also be used, but the above form of distilling apparatus is preferable. Measure in 500 c.c. of the water, boil, and distil off three tubes of 50 c.c. each.†

Test the last tube for ammonia by adding about 1 c.c. of

\* For preparation of this and other solutions used in water analysis, see p. 417.

† The ordinary Nessler tubes sold are somewhat clumsy. The writers, therefore, use tubes specially made of white glass, having a diameter of  $\frac{3}{4}$  inch and a length of 7 inches. These are flat-bottomed and are placed in a stand similar to those made for large test tubes, the tubes resting on a plate of glazed white porcelain. The tubes are marked at 50 c.c. The tubes of each set *must* have the same diameter.

Nessler solution, which will produce a slight yellow coloration if ammonia is present. Should any colour be produced, distil off another test tube, and again test. Repeat this, if necessary, until the last tube shows no coloration. Allow these tubes to stand. They contain the whole of the "free ammonia."

Add to the contents of retort 50 c.c. of alkaline permanganate of potash solution, continue distillation, and take off two tubes of 50 c.c. each—more if necessary. These tubes contain the "albuminoid ammonia."

Now make up a standard tube of ammonia by adding to a Nessler tube, marked not only at 50 c.c., but graduated at each 5 c.c., 5 c.c. of standard ammonia solution, and make up to the 50 c.c. mark with water free from ammonia; mix, add 1 c.c. of Nessler solution, and again mix.

To each of the "free" and "albuminoid" ammonia tubes (save those already treated) add 1 c.c. of Nessler solution, and after mixing allow to rest for five minutes. The liquid will now have acquired a yellow or brown colour, varying with the amount of ammonia present.

To estimate this—

Take the first free ammonia tube, and place by the side of the standard tube. If the colour in the standard tube is too deep, withdraw sufficient solution so that on looking down the tubes on to a white plate (raise the tubes a little from plate) the colours are of exactly the same depth or intensity. Note the amount of standard required. Repeat this with each tube, both of free and albuminoid ammonia. If the colour is darker than that of the standard, a fresh standard of double strength may be prepared.

Add together the cubic centimetres of standard required for the free and for the albuminoid ammonia, and calculate result as shown. Express in parts per million.

*Example :—*

Standard tube contains 5 c.c. of standard ammonia (0.01 milligramme per cubic centimetre). The 50 c.c., therefore, contains 0.05 milligramme of ammonia.

Free ammonia—

|  |
|--|
| 1st tube took 40 c.c. Standard to equal tint.    |
| 2nd tube took 20 c.c. Standard to equal tint.    |
| 3rd tube took <i>nil</i> Standard to equal tint. |
| 60 c.c. total.                                   |

As 50 c.c. = 0.05 milligramme of ammonia, therefore, 0.06 milligramme is present in the 60 c.c. that is in 500 c.c. water, or 0.12 per 1,000 c.c. of water. Now 1,000 c.c. contains 1,000,000 milligrammes, therefore, milligrammes per 1000 c.c. = parts per million. The sample contains, therefore, 0.12 part per million free ammonia.

Albuminoid ammonia (calculated in same way)—

|  |
|--|
| 1st tube took 20 c.c. Standard to equal tint.    |
| 2nd tube took 10 c.c. Standard to equal tint.    |
| 3rd tube took <i>nil</i> Standard to equal tint. |
| 30 c.c. total.                                   |

30 c.c. = 0.03 milligramme of ammonia in 500 c.c., or 0.06 milligramme in 1,000 c.c. or parts per million.

Do not allow the tubes to stand more than five minutes before comparing colour, and care must be taken that no

ammonia fumes or sulphuretted hydrogen are present in the laboratory during distillation or titration.

### 3. Oxygen required to Oxidise Organic Matter.

This process depends upon the estimation of the amount of oxygen required to oxidise the organic and other oxidisable matters in a known volume of water, when slightly acidified with sulphuric acid. For this purpose a standard solution of potassic permanganate is used in excess. The amount of unchanged permanganate is, after a given time, ascertained by means of a solution of sodic hyposulphite by the help of the iodine and starch reaction.

If ferrous salts, nitrites, or sulphuretted hydrogen are present, these will also decompose the permanganate of potash, but whilst organic matters are only slowly oxidised, the bodies named affect the reagent almost immediately. It is thus possible by conducting two experiments, one after standing 15 minutes, the other after 4 hours, to ascertain both the reduction due to iron, etc., and that due to organic matter.

To perform the experiment :—

Carefully clean a stoppered bottle and measure in 200 c.c. of the water. (The bottle, flask, and pipette must all be carefully cleaned with sulphuric acid, and thoroughly rinsed.)

Now add 10 c.c. of standard permanganate of potash solution,\* and 10 c.c. of dilute sulphuric acid, to which enough permanganate of potash has been added to render it of a faint pink colour.

\* See p. 413.



(A stock solution of 1 of acid + 3 of water may be prepared. To this enough permanganate of potash is added to impart a permanent pink tint.)

Stand for four hours, at about 80° F.

The undecomposed permanganate is then determined as follows :—

Fill a burette with the standard hyposulphite solution. Add to the water in the bottle a few drops of potassic iodide solution—sufficient to change the pink colour of the permanganate to yellow—due to liberation of free iodine. Run in hyposulphite solution from burette until yellow colour is nearly—but not quite—destroyed. Now add to the bottle a few drops of a freshly-prepared solution of gelatinised starch, when a blue colour will appear. Continue the addition of the hyposulphite solution until the blue colour just disappears. Read off amount of hyposulphite used. As the hyposulphite solution is always liable to change, a blank experiment must be made to determine its value. To do this, take a clean bottle or flask, add about 200 c.c. of distilled water, and 10 c.c. each of the standard permanganate of potash solution and dilute sulphuric acid. Add at once potassic iodide, and titrate with hyposulphite solution as before. This gives the value of the hyposulphite solution.

Now conduct a further experiment with a fresh 200 c.c. of the water, with the addition of permanganate and acid, precisely in the same way as before, but allowing the whole to stand 15 minutes before commencing to titrate.

We can now calculate amount of oxygen absorbed from the three figures we have thus obtained.

*Example :—*

200 c.c. of water, treated as above, required 9·7 c.c. hyposulphite solution after four hours, and 10·6 c.c. after 15 minutes, whilst the blank experiment required 11·3 c.c.

As 11·3 c.c. equals 10 c.c. permanganate, 9·7 c.c. equals 8·6 c.c., and  $10 - 8·6 = 1·4$ , the quantity of permanganate solution decomposed in four hours.

Similarly, as 11·3 equals 10 c.c. of permanganate, 10·6 equals 9·4 c.c., and  $10 - 9·4 = 0·6$  absorbed in 15 minutes.

Now, each cubic centimetre of the standard permanganate solution equals 0·1 milligramme of available oxygen, so that the above figures correspond to 0·14 and 0·06 milligramme respectively, or 0·07 and 0·03 per 100 c.c. of water.

Results by this test are usually expressed in terms of parts per 100,000, and, as 100 c.c. contains 100,000 milligrammes, we have the following result :—

Oxygen absorbed in  $\frac{1}{4}$  hour ... 0·03 part per 100,000.

Oxygen absorbed in 4 hours ... 0·07 part per 100,000.

In carrying out this test the hyposulphite solution must be standardised every day of using, but the permanganate of potash, if it has been prepared with a pure salt, will remain unchanged for a long time.

## ANALYTICAL EXAMINATION—INORGANIC.

### 4. Total Saline Residue—Sulphated.

Carefully ignite a perfectly clean platinum dish, place under the dessicator, and accurately weigh when just cold.

Measure into a graduated flask 250 c.c. of the water to be examined, and evaporate this over the boiling water bath, keeping the dish constantly filled up until the whole of the water has been evaporated.

If the water is suspected to contain a heavy quantity of salts in solution, such as, for example, a water treated for brewing purposes, it is more advantageous to use a less quantity, in which case 200 c.c., or sometimes even 100 c.c., will be found to be sufficient.

A few drops of dilute sulphuric acid are added to the water in the dish during evaporation, in order to convert all the salts naturally present in the water into the form of sulphates. By this means accurate weighing of the saline residue is much facilitated, and more reliable results are obtained than if the residue be dried without the addition of acid, since some salts part with their water of crystallisation with considerable difficulty at the high temperature required, and cannot therefore be ignited without loss due to decomposition or volatilisation.

After evaporation is completed, the dish is placed inside the boiling water bath for an hour and afterwards ignited, at first gently but afterwards more strongly, in order to drive off all excess of the sulphuric acid, a stage easily recognised by the absence of all white fumes. The dish with the residue is then cooled in the desiccator and weighed. The weight thus found is due to the total salts expressed as sulphates, and if 250 c.c. of the water have been taken, the weight found, multiplied by 0.4, will give the solid matter in 100 c.c., and this again, multiplied by 0.7, gives that in 70 c.c.

Now, one gallon of water weighs 70,000 grains, and 70 c.c. of water weighs 70,000 milligrammes, so that 70 c.c. of water is a sort of miniature gallon, wherein each milligramme corresponds to one grain. If we know the number of milligrammes of residue which 70 c.c. of water leave, we know the number of grains of solids in a gallon of water.

*Example :—*

|  | Grammes. |
|--|----------|
| Weight of dish and residue from 250 c.c. |          |
| water ... ..                             | 36·583   |
| Weight of empty dish ... ..              | 36·410   |
|  | <hr/>    |
|  | 0·173    |
|  | <hr/>    |

Then  $0·173 \times 0·4 = 0·0692$

$0·0692 \times 0·7 = 0·0484$  gramme or 48·4 milligrammes  
sulphated solids per 70 c.c.

The water contains, therefore, 48·4 grains per gallon of total sulphated residue.

## 5. Lime.

Place 500 c.c. of water in a large beaker, and add a few drops of hydrochloric acid. Boil down to half its bulk, then add excess of ammoniac oxalate solution (about 5 c.c.), Add about the same quantity of ammoniac chloride and ammoniac hydrate, when the lime separates in the form of a white precipitate of calcic oxalate. The liquid should be gently warmed, then set aside, and allowed to stand for not less than three hours, by which time the precipitation will be complete, and the supernatant liquid bright. The liquid is now very carefully decanted in successive quantities on

to a filter previously moistened with water, until the whole of the clear liquid has passed through the filter. The insoluble calcic oxalate and the small quantity of liquid left in the beaker are now stirred up by means of a feather, or a glass rod to the end of which is attached a short piece of indiarubber tubing, and the liquid and precipitate are transferred to the filter. All the precipitate is carefully washed on to filter with successive small quantities of water, any particles adhering to the glass being rubbed off with the rod or feather, so that at the completion of the operation not a particle is left in the beaker. The filter paper is now washed with boiling distilled water, and, when thoroughly drained, placed in the funnel jacket of water bath to dry. Should the liquid not have been bright as it passed through the filter, it must be again and again returned until perfectly brilliant. There is but seldom any difficulty, however, about this matter in the filtration of a lime precipitate. The object of the addition of ammoniac hydrate is as follows\* :—Lime may be precipitated in neutral or even faintly acid solution by ammoniac oxalate, but the precipitation is in those cases not complete ; in ammoniacal solutions, however, the precipitation is perfect. The addition of ammoniac chloride is quite unnecessary for the separation of the calcic salts, but is added to prevent the simultaneous precipita-

\* Fresenius has shown that in the presence of large quantities of magnesia, this process is not rigidly accurate, but that a small quantity of this body precipitates along with the lime, and further, that unless a large excess of ammoniac oxalate be present the lime is not completely precipitated. He considers, therefore, a re-solution and precipitation of the calcic oxalate a necessity, but it is seldom that a brewing water contains sufficient magnesia to render such an operation imperative.

tion of magnesia, which also forms an insoluble oxalate, not, however, in the presence of ammoniac chloride. A quantity of oxalate of ammonia should be added, sufficient not only to convert the calcic but also the magnesian salts into oxalate.

The filtrate from the lime is required for the subsequent estimation of magnesia, and is therefore set aside. The precipitate having now been dried in the funnel jacket, it remains to ignite and weigh it. This may be accomplished by either of two methods, depending upon the amount of precipitate obtained. If the quantity be very small, so that it can scarcely be discerned on the filter paper, it is folded and ignited over a tared platinum crucible, being held by a piece of platinum wire fixed in the end of a glass rod, and some five or six inches in length, one end of which is twisted round the paper. When the paper has burnt white or grey, it is allowed to fall into the crucible, which is strongly heated for some time, and afterwards the heat is still further raised by means of a blowpipe. This effects the reduction of the calcic oxalate to calcic carbonate, and finally to calcic oxide. The crucible and contents are weighed, the ash of filter paper deducted, and the results calculated to grains per gallon by dividing by 5 and multiplying the milligrammes so found by 0.7. As the lime was weighed as such, no other calculation is needed in this case.

Should the quantity of calcic oxalate have been considerable, however, it is difficult to drive off the whole of the carbonic acid by ignition, so as to convert the precipitate into calcic oxide ( $\text{CaO}$ ), and it is for this reason advisable to weigh the precipitate as calcic carbonate. A



very slight heat is sufficient to decompose calcic oxalate into carbonate ; but there is this difficulty, that small quantities of carbonic acid are very readily disengaged from the carbonate. By careful treatment it is, nevertheless, possible to apply a small Bunsen flame in such a manner as to drive off the carbon monoxide from the oxalate, and to leave carbonate of lime, without any reduction to the state of oxide. The change from oxalate to carbonate may be known by the precipitate assuming a greyish hue, while frequently the carbon monoxide evolved burns over the precipitate with a bluish flame. As however, skilful manipulation is required to effect this without losing any carbon dioxide, it is almost always advisable to "re-carbonate." This operation consists of the addition of a little ammoniac carbonate solution—which must be quite pure, and, when heated, volatilise without leaving any residue—to the precipitate, then carefully and thoroughly drying on the water bath, very gently heating for a moment or two over a small Bunsen flame, and weighing when cool. By this means any carbonic acid which may have been lost is replaced at the expense of the ammoniac carbonate, while the excess of the latter is volatilised on drying in the water bath, and subsequent exposure to a gentle heat over a flame. This re-carbonation should be repeated until a constant weight is obtained. After deducting the ash of filter paper, the amount of  $\text{CaCO}_3$  is calculated to that of  $\text{CaO}$ . Now the atomic weight of  $\text{CaCO}_3$  is 100, and that of  $\text{CaO}$  is 56 ; it follows, therefore, that 100 parts of  $\text{CaCO}_3$  contain 56 parts of  $\text{CaO}$ . The quantity of calcic oxide is calculated by a simple sum :—



As 100 : 56 :: amount of  $\text{CaCO}_3$  found :  $\text{CaO}$ .

Or the same thing is effected by multiplying the  $\text{CaCO}_3$  found by 0.56.

*Example :—*

500 c.c. of water gave after re-carbonation of precipitate :—

|                          |     |     |     | Grammes. |
|--------------------------|-----|-----|-----|----------|
| Crucible and precipitate | ... | ... | ... | 19.989   |
| Crucible alone           | ... | ... | ... | 19.801   |
|                          |     |     |     | <hr/>    |
|                          |     |     |     | 0.188    |
|                          |     |     |     | <hr/>    |

Deducting 0.002 for ash of filter paper, we have a net weight of 0.186 gramme  $\text{CaCO}_3$ .

Then  $0.186 \times 0.2 = 0.0372$  gramme in 100 c.c.

and  $0.0372 \times 0.7 = 0.02604$  gramme of  $\text{CaCO}_3$  in 70 c.c. or  
26.04 grains per gallon as  $\text{CaCO}_3$ .

Then  $26.04 \times 0.56 = 14.58$  grains per gallon of lime ( $\text{CaO}$ ).

## 6. Magnesia.

The filtrate from the lime is treated for magnesia, which is precipitated by the addition of ammonium phosphate (the soda salt may be substituted) and strong ammonium hydrate until the liquid smells pungently; about one-fifth of the total volume of the liquid is sufficient. The liquid should then be stirred continuously for about ten minutes, and allowed to stand for half an hour, as under these conditions the whole of the magnesia is precipitated, and may at once be filtered off. If not continuously stirred in this way the precipitate should be allowed to stand for six hours. In either case the precipitate is then filtered, washed well

with weak ammonium hydrate (one of strong ammonia with three of water), dried, and burnt off in a platinum crucible. Scrape as much as possible into the crucible with a platinum wire, let the ash drop in, and ignite strongly for about five minutes with the cover half over. The precipitate should be grey in colour, and is weighed as pyro-phosphate of magnesia,  $2(\text{MgO}).\text{P}_2\text{O}_5$ .

The atomic weight of  $2(\text{MgO}).\text{P}_2\text{O}_5$  being 222, and that of  $2\text{MgO}$  being 80, it follows that 222 parts of pyro-phosphate of magnesia correspond to 80 parts of magnesia; or the factor 0.36 may be employed to convert  $2(\text{MgO}).\text{P}_2\text{O}_5$  into  $2\text{MgO}$ .

*Example :—*

The precipitate from magnesia estimation gave :—

|                          |     |     |     | Grammes. |
|--------------------------|-----|-----|-----|----------|
| Crucible and precipitate | ... | ... | ... | 16.480   |
| Crucible alone           | ... | ... | ... | 16.455   |
|                          |     |     |     | <hr/>    |
|                          |     |     |     | 0.025    |
|                          |     |     |     | <hr/>    |

Deducting 0.002 for ash of paper, there remains 0.023  $2(\text{MgO}).\text{P}_2\text{O}_5$ .

Then  $0.023 \times 0.2 = 0.0046$  per 100 c.c. or 0.00322 per 70 c.c.

Then  $3.22 \times 0.36 = 1.16$  grains per gallon magnesia ( $\text{MgO}$ ).

## 7. \*Alkalies.—Soda and Potash.

Place 500 c.c. of the water in a beaker, and boil down to about 25 c.c. Add barium hydrate in slight excess (about

\* The direct determination of the alkalies is a matter of some trouble and difficulty, so that when it is not essential to estimate the

10 c.c.), and allow to stand in warm place (on water bath) for one hour. Filter and wash well with hot water (precipitate not required). To filtrate, add ammoniac chloride solution (about 5 c.c.) and ammoniac carbonate solution in excess—probably about 10 c.c., but until no more barium is thrown down. The barium precipitates all the bases, except soda and potash, and the ammoniac chloride and carbonate precipitate the excess of barium, and leave the alkalies in solution as chlorides.

After addition of ammoniac carbonate as above, allow to stand for one or two hours, then filter, and wash with hot water (precipitate not required). Boil filtrate down to about 25 c.c., transfer to a clean platinum dish, and evaporate to dryness. When thoroughly dry (not before), gently ignite until fumes of ammonia salts cease to come off; cool, weigh, again gently heat, and repeat weighing. Care must be taken not to ignite the residue too strongly, or small quantities of chlorides may be volatilised. When constant, dissolve residue in a small quantity of hot water, and filter through a small paper into a small porcelain dish. Wash with a few drops of water, return the paper to the platinum dish, ignite, and weigh.

The weight thus obtained is the tare of the dish, the ash of the filter, and traces of insoluble substances accidentally present with the alkalies. This deducted from the previous weighing gives the alkalies as chlorides.

It now remains to separate the soda and potash. To do

potash present in the water (the potash usually being present only as a trace) the alkalies may be determined indirectly as described on p. 283.

this, evaporate the above filtrate in the porcelain dish to dryness over the water bath, add about 5 c.c. of platinum bichloride, and again evaporate to dryness. When cool, cover the precipitate with alcohol (about 70 per cent.), and let stand for four hours. After standing, the alcohol should be distinctly yellow in colour. If this is not so, the alcohol must be evaporated off on the steam bath, and a small further quantity of bichloride of platinum solution added, then dried, and the treatment with alcohol proceeded with as before. In the meantime place a small (7 cm.) tared filter in a weighing bottle, dry in water oven, and weigh after cooling.

Filter the alcoholic solution through the tared paper, wash with alcohol, dry, return to weighing bottle, place this in oven for about 15 minutes, cool, and weigh. The gain in weight is due to potash existing as potassio-platinic chloride— $(\text{KCl})_2\text{PtCl}_4$ . To calculate to potassium chloride multiply by factor 0.307.\* Deduct the potassic chloride thus found from total chlorides, and the difference is sodium chloride.

The sodic chloride is converted into soda ( $\text{Na}_2\text{O}$ ) by multiplying by factor 0.53.†

The potassic chloride is converted into potash ( $\text{K}_2\text{O}$ ) by multiplying by factor 0.63.‡

\*  $(\text{KCl})_2 \text{PtCl}_4 = 484.5$  gives  $2\text{KCl} = 148.7$ , therefore  $1 = 0.307$ .

†  $2\text{NaCl} = 116.7$  gives  $\text{Na}_2\text{O} = 62.0$ , therefore  $1 = 0.53$ .

‡  $2\text{KCl} = 148.7$  gives  $\text{K}_2\text{O} = 94.0$ , therefore  $1 = 0.63$ .

*Example :—*

|                              |     | Grammes. |
|------------------------------|-----|----------|
| Platinum dish and residue... | ... | 40·920   |
| Dish and insoluble matter... | ... | 40·864   |
|                              |     | <hr/>    |
|                              |     | 0·056    |
|                              |     | <hr/>    |

Then

$$0·056 \times 0·2 = 0·0112, \text{ and } 0·0112 \times 0·7 = 0·00784.$$

Total chlorides, 7·84 grains per gallon.

Potash—

|                              |     | Grammes. |
|------------------------------|-----|----------|
| Tube, paper, and residue ... | ... | 14·069   |
| Tare of tube and paper ...   | ... | 14·049   |
|                              |     | <hr/>    |
|                              |     | 0·020    |
|                              |     | <hr/>    |

Then  $0·02 \times 0·2 = 0·004$ , and  $0·004 \times 0·7 = 0·0028$ .

Therefore potassio-platinic chloride = 2·80 grains per gallon. And  $2·80 \times 0·307 = 0·85$  potassic chloride.

|                       |     |     | Grains per gallon. |
|-----------------------|-----|-----|--------------------|
| Total alkalies ...    | ... | ... | 7·84               |
| Potassic chloride ... | ... | ... | 0·85               |
|                       |     |     | <hr/>              |
| Sodic chloride ...    | ... | ... | 6·99               |
|                       |     |     | <hr/>              |

$$\text{NaCl } 6·99 \times 0·53 = 3·70 \text{ Na}_2\text{O}.$$

$$\text{KCl } 0·85 \times 0·63 = 0·53 \text{ K}_2\text{O}.$$

The accurate estimation of soda and potash is not easy, as frequently, owing to incomplete separation of other bases, the alkalies are too high, and this error is thrown upon the soda, as the potash is subsequently determined directly.

## 8. Sulphuric Acid.

Take 500 c.c. of water, add a little hydrochloric acid (about 5 c.c. dilute), boil to half bulk, and add baric chloride solution in slight excess. Boil briskly for a minute or two, and allow to settle in a warm place. Filter\* and wash the precipitate thoroughly—especially round the top of the filter—with boiling distilled water.

Place the precipitate in the funnel jacket to dry, and when dry ignite in a tared platinum or porcelain crucible, as described under "Ignition." The ash of filter and the tare of crucible are deducted from the weight found, and the amount remaining multiplied by 0.2, which gives the sulphate of barium per 100 c.c.; this again multiplied by 0.7 indicates the amount per 70 c.c., and the milligrammes thus found equal the grains per gallon of baric sulphate. It now only remains to ascertain the quantity of sulphuric acid corresponding to the weight of baric sulphate obtained. The molecular weight of  $\text{BaSO}_4$  is 233, and that of sulphuric anhydride ( $\text{SO}_3$ ) is 80. 233 parts, therefore, of baric sulphate correspond to 80 parts of sulphuric anhydride; and a simple rule-of-three sum gives the amount of sulphuric acid in any known quantity of baric sulphate. In place of the calculation we may multiply by the factor 0.343.

\* Baric sulphate being a very finely divided precipitate, it is sometimes difficult to obtain a clear filtrate. A recommendation has been made to introduce into the liquid, immediately after the addition of the precipitant (baric chloride), a minute quantity of pure starch—0.005 gramme being sufficient; this has a conglomerating action upon the precipitate, securing its retention in the filter paper. No correction is necessary, for the saline matter thus introduced is practically *nil*.

*Example :—*

|                                   | Grammes. |
|-----------------------------------|----------|
| Crucible and precipitate ... ..   | 19·910   |
| Crucible alone ... ..             | 19·799   |
|                                   | <hr/>    |
|                                   | 0·111    |
| Deduct ash of filter paper ... .. | 0·001    |
|                                   | <hr/>    |
|                                   | 0·110    |
|                                   | <hr/>    |

Therefore, from 500 c.c. of the water we obtain 0·110 gramme of baric sulphate.

Then  $0·110 \times 0·2 = 0·022$ , and  $0·022 \times 0·7 = 0·0154$   $\text{BaSO}_4$ , or 15·40 grains per gallon.

This is converted into sulphuric anhydride by multiplying by 0·343. Thus  $15·40 \times 0·343 = 5·28$  grains per gallon  $\text{SO}_3$ .

## 9. Nitric Acid.

There are several methods which are applicable to the determination of nitric acid in water. The simplest, perhaps, is the indigo process, but the accuracy of the method is disturbed in the presence of much chlorides, and therefore an alternative method is also given.

INDIGO PROCESS.—The indigo process, although originally devised by Marx, was much improved and brought into prominent notice by Warrington.\* From its simplicity and rapidity, it is admirably adapted for use in water analysis, and, although objections have been urged against it, there can be no doubt of its utility when properly conducted.

\* *Jour. Chem. Soc. Trans.*, vol. 35, p. 578.



When dealing with very concentrated solutions, and in presence of much chlorides, its results are apt to be slightly irregular. The results by this method are almost invariably slightly below the truth, this error increasing with the strength of the nitric acid solution. In the writers' experience the drawback to the test is not in its actual performance—for that is easy—but the difficulty of preparing a satisfactory standard solution of indigo; this, however, it has been found possible to overcome.

To conduct the estimation, proceed as follows :—

Take 50 c.c. of the water, place it in an 8-ounce flask, and add to it an equal bulk of pure, strong sulphuric acid, allowing the acid to run down the sides of the flask, and avoiding mixing the solutions as far as possible. Now agitate the contents of the flask, and rapidly add the standard indigo solution from a burette until a permanent faint bluish-green colour is just perceptible. Read off the number of cubic centimetres consumed, and as each cubic centimetre corresponds to 0.1 milligramme of nitrogen, the amount of that substance in the 50 c.c. of water is found by multiplying the cubic centimetres of indigo used by 0.1. To find from this the corresponding amount of nitric acid is an easy matter. Two atoms of nitrogen ( $N_2$ ) are contained in the molecule of nitric anhydride ( $N_2O_5$ ); the calculation is, therefore, as below :—

$$N_2 = 28. \quad N_2O_5 = 108.$$

$$28 : 108 :: N \text{ found} : N_2O_5 \text{ wanted.}$$

Or by dividing the molecular weight of  $N_2$  into that of  $N_2O_5$  the factor 3.86 is obtained, which gives directly the amount

of nitric acid (anhydrous) from the nitrogen found. The results are calculated to grains per gallon in the manner already described.

*Example :—*

50 c.c. of water took 2.4 c.c. indigo solution.

$2.4 \times 2 = 4.8$  and  $4.8 \times 0.7 = 3.36$  c.c. per 70 c.c. water.

$3.36 \times 0.1 = 0.336$  grain per gallon of N.

$0.336 \times 3.86 = 1.29$  grains per gallon of nitric anhydride.

If, when titrating with indigo, more than 8 c.c. of standard is consumed, a fresh experiment must be made, using 25 c.c. of the water to be tested, and 25 c.c. of distilled water, of course making correction for this in the calculation.

**Crum's Method.**—As an alternative to the above process, the following method is described. This is known as Crum's method. It requires some knowledge of gas analysis, and is nothing like so easy of accomplishment as the indigo method. It is useful where the presence of much chlorine in the water interferes with the indigo process, as in the Crum method the hydrochloric acid gas disengaged is driven off before the process of decomposing the nitrate is reached, since the nitrate will only decompose after well mixing and in close contact with the mercury in the burette.

The following details of the process are taken from "Sutton's Volumetric Analysis," p. 468:—

A Lunge's nitrometer is charged with mercury, and the three-way stopcock closed, both to measuring tube and waste pipe. The concentrated filtrate is poured into the cup at the top of the measuring tube, and the vessel which contained it rinsed with 1 c.c. of water, and the contents added. The stopcock is opened to the measuring tube, and, by lowering the pressure tube, the liquid is drawn out of the cup into the tube. The basin is again rinsed with 5 c.c. of pure strong sulphuric acid, and this is also transferred to the cup and drawn into the measuring tube. The stopcock is once more closed, and 12 c.c. more sulphuric acid put into the cup, and the stopcock opened to the measuring tube until 10 c.c. of acid have

passed in. The excess of acid is discharged, and the cup and waste pipe rinsed with water. Any gas which has collected in the measuring tube is expelled by opening the stopcock and raising the pressure tube, taking care no liquid escapes. The stopcock is closed, the measuring tube taken from its clamp and shaken by bringing it slowly to a nearly horizontal position, and then suddenly raising it to a vertical one. This shaking is continued until no more gas is given off, the operation being, as a rule, complete in 15 minutes. Now prepare a mixture of one part of water with five parts of sulphuric acid, and let it stand to cool. After an hour pour enough of this mixture into the pressure tube to equal the length of the column of acidulated water in the working tube, bring the two tubes side by side, raise or lower the pressure tube until the mercury is at the same level in both tubes, and read off the volume of nitric oxide. This volume, expressed in cubic centimetres and corrected to normal temperature and pressure, gives, when multiplied by 0.175, the nitrogen in nitrates, in grains per gallon, if 250 c.c. of the water have been used.

## 10. Nitrous Acid.

A good test is that devised by Griess and depends upon the formation of a red dye obtained when sulphanilic acid together with naphthylamine hydrochloride is added to an acidified solution of a nitrite. The test is an extremely delicate one, and is performed as follows :—

100 c.c. of the water to be examined are measured into a Nessler jar and acidified with one drop only of concentrated hydrochloric acid. 1 c.c. of the sulphanilic acid is then added, followed by 1 c.c. of naphthylamine solution, the whole is then thoroughly mixed with a clean glass rod and allowed to stand for 30 minutes covered with a watch glass. The test may be made a quantitative one by using a standard solution of sodium nitrite\* and titrating

\* For preparation, see p. 422.

this against the water under examination, but it is rarely necessary to make the test other than a qualitative one. A slight pink coloration will indicate a trace of nitrous acid in the water, a deep rose colour indicates a distinct trace of the nitrous acid. It has already been mentioned that the test is an extremely sensitive one, and waters are rarely found showing other than what is an extremely small amount indicated in this way. The average amount found in a water would not be likely to exceed 2 to 4 parts of nitrogen in the form of nitrites per 100,000,000 parts of water.

## II. Chlorine.

Take 100 c.c. of the water in a beaker, place on white slab, and add a few drops of potassium chromate solution (it is, of course, essential that this should be quite free from chlorides). Add now, from a burette, standard solution of silver nitrate (1 c.c. of which corresponds to 1 milligramme of chlorine) until the reddish colour imparted to the liquid from formation of silver chromate, which at first disappears when the liquid is stirred, just becomes permanent. Read off the number of cubic centimetres taken, and multiply this by 0·7, which will give the milligrammes of chlorine in 70 c.c., or grains per gallon.

As an example, 100 c.c. of a water required 6·1 c.c. of silver nitrate to impart a permanent reddish tint. Then 6·1 c.c. of silver nitrate solution equals 6·1 milligrammes of chlorine, therefore  $6·1 \times 0·7 = 4·27$  milligrammes of chlorine per 70 c.c., or grains per gallon.

The theory of the reaction with silver nitrate is as follows:—Silver has an affinity for both chlorine and chromic acid ; but its affinity for the former is much greater than for the latter, and hence no silver chromate is formed until the whole of the chlorine has been combined with the silver. The appearance of the reddish-brown colour, therefore, indicates that the whole of the chlorine has combined with the silver, and that the chromic acid in the potassium chromate is beginning to combine also ; it is therefore necessary to discontinue the addition of the silver nitrate the instant that a permanent reddish-brown colour is imparted to the liquid. Students often carry the addition of silver solution too far ; it should be stopped as a permanent faint coloration is obtained.

## 12. Alkalinity before and after Boiling.

350 c.c. of water is boiled for three-quarters of an hour, then filtered through a filter paper, previously moistened with boiling distilled water, the flask once rinsed and the washings transferred to the filter. The filtrate after cooling is titrated with decinormal acid,\* adding a few drops of methyl orange as an indicator, and continuing the addition of the acid until a very faint pink coloration is produced. Read off cubic centimetres required.

The titration of the boiled water being effected, another 350 c.c. of water is measured out and titrated without any previous treatment. The difference between the two titrations is due to the precipitation on boiling of carbonate of lime, with perhaps a trace of carbonate of magnesia. This

\* See Standard Solutions, p. 408.

depends on the well-known fact that carbonate of lime is almost insoluble in water, but it is dissolved as a bi-carbonate in the presence of free carbonic acid ; on heating, the  $\text{CO}_2$  is expelled, the carbonate of lime is thrown out of solution, and removed by filtration. This substance is not, as has been observed, absolutely insoluble in boiling water, but remains in solution to the extent of 1.26 grains per gallon, and this figure must be taken into account when calculating out the result of this and the subsequent operation. The difference between the two titrations indicates, therefore, the carbonate of lime removed by boiling, and if, as recommended, 350 c.c. of water are employed, each cubic centimetre of decinormal acid corresponds to one grain of carbonate of lime per gallon.

*Example :—*

350 c.c. of raw water required 21.5 c.c. of N/10 acid.

350 c.c. of boiled and filtered

|                |     |     |     |   |   |
|----------------|-----|-----|-----|---|---|
| water required | ... | ... | 1.6 | „ | „ |
|----------------|-----|-----|-----|---|---|

|                               |      |   |   |
|-------------------------------|------|---|---|
| Alkalinity removed by boiling | 19.9 | „ | „ |
|-------------------------------|------|---|---|

or grains per gallon as  $\text{CaCO}_3$ .

To this figure add 1.26 (solubility of  $\text{CaCO}_3$  in boiled water).

Thus  $19.9 + 1.26 = 21.16$  grains per gallon of  $\text{CaCO}_3$ .

This estimation is an excellent check on the accuracy of other analytical results, particularly with waters containing carbonate of soda.



### 13 and 14. Lead and other Heavy Metals, and Iron.

500 c.c. of the water is taken, a few drops of hydrochloric acid added, and the liquid boiled down to a bulk of about 20 c.c. It is then transferred to a test tube, and a little freshly prepared sulphuretted hydrogen water added. If a black or brown coloration is produced, lead, copper, or other heavy metal is present. Now add ammonium hydrate until the solution, after mixing, smells distinctly of ammonia. If a further black or brown coloration is produced iron is present. It is very unusual to get any coloration on adding sulphuretted hydrogen to the acid solution, lead, etc., being of rare occurrence, and if blackening occurs it is better to repeat the test before certifying the presence of these metals.

In the event of their absence—no coloration having occurred—there is generally some colour on addition of ammonia, for most waters contain some iron. The proportion of these may be judged by the depth of colour produced.

The iron may, if desired, be quantitatively estimated by taking 100 c.c. of water, adding a little bromine water, acidifying with a few drops of hydrochloric acid and boiling down to half bulk. The iron, if present, is then estimated exactly as described under sugar analysis, p. 205.

### 15. Silica.

This substance is not usually determined, as it has no influence upon the value of the water, either for brewing or other purposes. It may, however, be estimated by



evaporating to dryness 500 c.c. of water, treating the residue with hydrochloric acid, evaporating again, and igniting. The silica is thus rendered insoluble, and, upon a subsequent treatment of the residue with hydrochloric acid and hot water, is left, while all other substances are dissolved. The solid residue after treatment for the saline residue may be used for this estimation. The silica is thrown upon a filter, well washed, and dried ; it is afterwards ignited in the usual manner and weighed as silica.

### 16. Carbonate of Soda.

The determination of this salt is by no means easy, but the following are the data upon which a sufficiently accurate conclusion may be arrived at as to its presence or absence :—Should there be an alkalinity after boiling above that naturally present (1.26 grains), it must be due to magnesian or sodic carbonate. The determination of the magnesia, as before mentioned, will show whether the former is the case ; for if, after calculating out the results and combining bases with acids, an excess of magnesia be left, this—combined with an alkalinity after boiling—points to the presence of carbonate of magnesia. When, however, there is no excess of magnesia, the alkalinity after boiling indicates carbonate of soda.

## COMBINATION OF THE ACIDS AND BASES.

Having ascertained the amounts of the above, we have now to calculate the salts into the form in which they exist in the water sample.

And here we meet with some difficulty, for although we can say with certainty what amounts of bases and acids are present, we are not so sure of the combination in which they exist, and thus it is that, given the same amount of bases and acids, the statement of the salts and their amounts may vary in reports formulated by different analysts. The salts are, however, generally calculated on the basis proposed by Fresenius, though with some modification in particular cases :—

The following general rules may be followed :—

1. The chlorine is combined with sodium.
2. If chlorine is then left over (often these amounts just saturate each other), combine it with calcium, magnesium, and potassium in the order named.
3. If after combination of the chlorine there is still an excess of sodium, combine it as soda with sulphuric acid, strictly, sulphuric anhydride ( $\text{SO}_3$ ). First, however, combine any potash with sulphuric acid, which acid is only available for soda provided the potash has already been satisfied. If soda still remains, combine it with nitric acid, strictly, nitric anhydride ( $\text{N}_2\text{O}_5$ ); if there is then still excess of soda, combine with carbon dioxide.
4. Combine lime first with nitric acid, then with sulphuric acid (the potash having been first satisfied as above), finally with carbon dioxide. If chlorine has been in excess of that required to saturate the sodium, a portion of the calcium may have been used to combine with such excess.
5. Combine magnesia first with nitric acid (if lime has not saturated it), then with sulphuric acid. Any excess is combined with carbon dioxide.

It will be noted that any excess of base is always finally combined with carbon dioxide, no determination of which has been included in the analysis. The reason for such combination is the fact that every ordinary water contains free carbonic acid, and, therefore, any excess of bases must exist as carbonates.

The sum of these carbonates is checked by the alkalinity of the water, and if, by calculation, carbonate of soda or potash is found to exist, its presence should have been also indicated by the alkalinity after boiling.

The sum of the salts when each is calculated to its equivalent sulphate should closely agree with the total sulphated saline residue obtained on p. 259.

## CALCULATION OF WATER RESULTS.

Results of analysis, so far, are as follows :—

|  |     |     |     |     | Grains per gallon. |
|--|-----|-----|-----|-----|--------------------|
| Sulphated saline residue                   | ... | ... | ... | ... | 48.40              |
| Lime                                       | ... | ... | ... | ... | 14.58              |
| Magnesia                                   | ... | ... | ... | ..  | 1.16               |
| Soda                                       | ... | ... | ... | ... | 3.70               |
| Potash                                     | ... | ... | ... | ... | 0.53               |
| Sulphuric anhydride                        | ... | ... | ... | ... | 5.28               |
| Nitric anhydride                           | ... | ... | ... | ... | 1.29               |
| Chlorine                                   | ... | ... | ... | ... | 4.27               |
| Total alkalinity expressed as carbonate of |     |     |     |     |                    |
| lime                                       | ... | ... | ... | ... | 21.5               |

Combine the sodium with chlorine (to form chloride of sodium).

It will be observed that in our analytical figures the

sodium (Na) is expressed as soda ( $\text{Na}_2\text{O}$ ). We must, therefore, calculate this back to sodium.

Now, 62 parts of  $\text{Na}_2\text{O}$  contain 46 parts of Na.

$$\text{Then } 3.70 \text{ (amount of } \text{Na}_2\text{O}) \times \frac{46}{62} = 2.74 \text{ Na.}$$

To combine this with the chlorine :—

23 parts Na require 35.5 parts of Cl.

$$\text{Then } 2.74 \text{ (amount of Na)} \times \frac{35.5}{23} = 4.23 \text{ Cl required,}$$

$$\text{and } 2.74 \text{ Na} + 4.23 \text{ Cl} = 6.97 \text{ NaCl.}$$

We have now used up both soda and chlorine.

Combine potash with sulphuric anhydride (to form  $\text{K}_2\text{SO}_4$ ).

94 parts of  $\text{K}_2\text{O}$  require 80 parts of  $\text{SO}_3$ .

$$\text{Then } 0.53 \text{ (amount of } \text{K}_2\text{O}) \times \frac{80}{94} = 0.45 \text{ SO}_3 \text{ required,}$$

$$\text{and } 0.53 \text{ K}_2\text{O} + 0.45 \text{ SO}_3 = 0.98 \text{ K}_2\text{SO}_4.$$

Combine nitric anhydride with lime (to form  $\text{Ca}(\text{NO}_3)_2$ ).

108 parts  $\text{N}_2\text{O}_5$  require 56 parts of CaO.

$$\text{Then } 1.29 \text{ (amount of } \text{N}_2\text{O}_5) \times \frac{56}{108} = 0.67 \text{ CaO required}$$

$$\text{and } 0.67 \text{ CaO} + 1.29 \text{ N}_2\text{O}_5 = 1.96 \text{ Ca}(\text{NO}_3)_2.$$

Combine remaining sulphuric anhydride with lime.

We have already used 0.45 of  $\text{SO}_3$  to satisfy the  $\text{K}_2\text{O}$ .

$$\text{Total SO}_3 = 5.28 - 0.45 = 4.83 \text{ remaining SO}_3.$$

80 parts  $\text{SO}_3$  require 56 parts of CaO.

$$\text{Then } 4.83 \text{ (remainder of SO}_3) \times \frac{56}{80} = 3.38 \text{ CaO,}$$

$$\text{and } 4.83 \text{ SO}_3 + 3.38 \text{ CaO} = 8.21 \text{ CaSO}_4.$$

Combine remaining lime with carbonic acid.

We have used 0.67 CaO for combination with  $\text{N}_2\text{O}_5$  and 3.38 CaO for combination with  $\text{SO}_3$  = a total of 4.05.

This deducted from the total CaO in the water gives :—

14.58 (CaO total) - 4.05 (already used) = 10.53 CaO remaining.

56 parts of CaO require 44 parts of  $\text{CO}_2$ .

Then  $10.53$  (remainder of CaO)  $\times \frac{44}{56} = 8.27 \text{ CO}_2$ ,

and  $10.53 \text{ CaO} + 8.27 \text{ CO}_2 = 18.80 \text{ CaCO}_3$ .

Combine magnesia with carbonic acid.

(There are no acids as determined directly left to combine with the magnesia, which must, therefore, exist as carbonate.)

40 parts of MgO require 44 parts of  $\text{CO}_2$ .

Then  $1.16$  (amount of MgO)  $\times \frac{44}{40} = 1.28 \text{ CO}_2$ ,

and  $1.16 \text{ MgO} + 1.28 \text{ CO}_2 = 2.44 \text{ MgCO}_3$ .

The amount of carbonates as arrived at by calculation may be checked by comparison with the "alkalinity" of the water. The alkalinity is expressed as carbonate of lime, and, therefore, if other carbonates are present they must be calculated into terms of carbonate of lime.

Thus the carbonate of lime has been found to be 18.80 ; but we have also 2.44 grains of carbonate of magnesia. Now 88 parts of  $\text{MgCO}_3$  have the same alkalinity as 100 parts of  $\text{CaCO}_3$ .

Then  $2.44 \times \frac{100}{88} = 2.77 \text{ as } \text{CaCO}_3$

Adding the actual  $\text{CaCO}_3$  to the  $\text{CaCO}_3$  equivalent to the  $\text{MgCO}_3$  we obtain 21.57 grains of alkalinity as against 21.5.

The composition of the water may therefore be stated as follows :—

|  |     | Grains per gallon. |
|--|-----|--------------------|
| Chloride of sodium ( $\text{NaCl}$ )           | ... | 6.97               |
| Sulphate of potash ( $\text{K}_2\text{SO}_4$ ) | ... | 0.98               |
| Sulphate of lime ( $\text{CaSO}_4$ )...        | ... | 8.21               |
| Nitrate of lime, $\text{Ca}(\text{NO}_3)_2$    | ... | 1.96               |
| Carbonate of lime ( $\text{CaCO}_3$ )          | ... | 18.80              |
| Carbonate of magnesia ( $\text{MgCO}_3$ )      | ... | 2.44               |
|  |     | <hr/>              |
| Total saline residue                           | ... | 39.36              |
|  |     | <hr/>              |

**To Check the Accuracy of the Analysis.**—Convert the above salts each into its equivalent sulphate.

$$6.97 \text{ NaCl} \times \frac{\text{Na}_2\text{SO}_4}{2\text{NaCl}} = \frac{142}{117} \text{ or } 1.21 = 8.43 \text{ Na}_2\text{SO}_4,$$

$$1.96 \text{ Ca}(\text{NO}_3)_2 \times \frac{\text{CaSO}_4}{\text{Ca}(\text{NO}_3)_2} = \frac{136}{164} \text{ or } 0.83 = 1.63 \text{ CaSO}_4,$$

$$18.80 \text{ CaCO}_3 \times \frac{\text{CaSO}_4}{\text{CaCO}_3} = \frac{136}{130} \text{ or } 1.36 = 25.57 \text{ CaSO}_4,$$

$$2.44 \text{ MgCO}_3 \times \frac{\text{MgSO}_4}{\text{MgCO}_3} = \frac{120}{84} \text{ or } 1.43 = 3.49 \text{ MgSO}_4.$$

|  | Grains per gallon. |
|--|--------------------|
| Chloride of sodium expressed as sulphate | 8.43               |
| Sulphate of potash                       | 0.98               |
| Sulphate of lime                         | 8.21               |
| Nitrate of lime expressed as sulphate    | 1.63               |
| Carbonate of lime                        | 25.57              |
| Carbonate of magnesia                    | 3.49               |
|  | <hr/>              |
|  | 48.31              |
|  | <hr/>              |

The sulphated residue directly determined was found to be 48·40 grains per gallon, so that the salts determined as above, converted into sulphates and added together, give a total closely approximating to this figure ; the analysis, therefore, may be considered as accurately carried out.

**Method of Calculation when the Alkalies are Indirectly Determined.**—It has already been pointed out that a correct estimation of the alkalies is attended with some difficulty, and where it is not required to estimate the potash it is quite safe to determine the alkalies expressed as soda by difference. The calculations are then made as follows :—

The bases lime and magnesia, whose amounts in the water have been already determined, are first converted into their equivalent sulphates, and their total is subtracted from the sulphated residue obtained, the result being the alkalies present in the water expressed as sulphates.

Thus, for example, the results of analysis have given the following figures :—

|                                 | Grains per gallon. |
|---------------------------------|--------------------|
| Sulphated saline residue ... .. | 48·50              |
| Lime ... ..                     | 14·58              |
| Magnesia ... ..                 | 1·16               |

Now convert the lime into calcium sulphate.

56 parts CaO are equivalent to 136 parts CaSO<sub>4</sub>

$$\therefore 14\cdot58 \times \frac{136}{56} \text{ (or } 2\cdot43) = 35\cdot43 \text{ CaSO}_4$$

Similarly 1·16 parts of MgO  $\times \frac{120}{40}$  (or 3·0) = 3·48 MgSO<sub>4</sub>

38·91

The total sulphated residue was found to be 48·50 grains per gallon.

$\therefore 48\cdot50 - 38\cdot91 = 9\cdot59$  grains per gallon of total alkalies expressed as sulphates, and  $9\cdot59 \text{ Na}_2\text{SO}_4 \times \frac{\text{Na}_2\text{O}}{\text{Na}_2\text{SO}_4} = \frac{62}{146}$  (or 0·436) = 4·09 grains per gallon of Na<sub>2</sub>O.

Then proceed with the combination of bases and acids as above described, neglecting in this case the potash.

Even if the alkalies have been directly determined by analysis, as described on p. 264, a useful check on the results may be obtained



by calculating the bases lime and magnesia as sulphates as just described, converting also the soda and potash actually determined into their equivalent sulphates, and adding these salts together, when, if a correct analysis has been made, the sum of these four salts should be closely equivalent to the sulphated saline residue. Thus, for example :—

The soda found by direct determination was 3·70 grains per gallon, and the potassium 0·53 grains per gallon. To convert these into their equivalent sulphates we proceed as follows :—

$$3\cdot70 \text{ parts Na}_2\text{O} \times \frac{142}{62} \text{ (or } 2\cdot30) = 8\cdot50 \text{ Na}_2\text{SO}_4$$

$$0\cdot53 \text{ parts K}_2\text{O} \times \frac{174}{94} \text{ (or } 1\cdot85) = 0\cdot98 \text{ K}_2\text{SO}_4$$

The sulphates of lime and magnesia have already been estimated, so that we now have

|                                 |     |     |     | Grains per gallon. |
|---------------------------------|-----|-----|-----|--------------------|
| CaSO <sub>4</sub>               | ... | ... | ... | 35·43              |
| MgSO <sub>4</sub>               | ... | ... | ... | 3·48               |
| Na <sub>2</sub> SO <sub>4</sub> | ... | ... | ..  | 8·50               |
| K <sub>2</sub> SO <sub>4</sub>  | ... | ... | ... | 0·98               |
|                                 |     |     |     | <hr/> 48·39 <hr/>  |

Adding these results we get, therefore, 48·39, which compares very closely with 48·50, as obtained by the direct determination of the sulphated saline residue.

We may in this way conclude that, so far, the results are satisfactory, and that the determination of the alkalies has been accurate.

**Silicates.**—It should be mentioned that a possible source of error may arise in the determination of the sulphated residue, due to the presence of silica, should this substance be dissolved in the water in any considerable quantity as soluble silicate, although such a case rarely occurs, whilst it is obvious that an indirect determination of the alkalies is dependent not only on the correct estimation of the sulphated saline residue but also on that of lime and magnesia, and an error in the figure obtained for any of these results will give an incorrect figure for the alkalies.

The accuracy of the results obtained in this way is, however, checked by the alkalinity, and the method of indirect alkali determination is, on the whole, quite reliable, and very considerably lessens

the labour of water analysis, whilst potash salts are usually present in water only in very small quantities, and it is unnecessary, as a rule, to actually determine their amount except in those waters which are known to be treated with potash salts when added (for example, as kainit) to the mashing liquor.

*Second Example. Carbonate of Soda Water. Figures of Analysis :—*

|                         |     |     |     |     | Grains per gallon. |
|-------------------------|-----|-----|-----|-----|--------------------|
| Total sulphated residue | ... | ... | ... | ... | 34·50              |
| Lime ...                | ... | ... | ... | ... | 5·72               |
| Magnesia                | ... | ... | ... | ... | 2·41               |
| Soda ...                | ... | ... | ... | ... | 6·29               |
| Potash                  | ... | ... | ... | ... | 0·19               |
| Sulphuric anhydride...  | ... | ... | ... | ... | 0·77               |
| Nitric anhydride        | ... | ... | ... | ... | <i>nil</i>         |
| Nitrous acid ...        | ... | ... | ... | ... | <i>nil</i>         |
| Chlorine                | ... | ... | ... | ... | 2·66               |

The soda being in excess we take the chlorine and combine it with sodium.

Now 35·5 parts of Cl require 23 parts of Na.

Therefore  $2·66 \times \frac{23}{35·5} = 1·72$  Na required,

and  $1·72 \text{ Na} + 2·66 \text{ Cl} = 4·38 \text{ NaCl}$ ,

and  $1·72 \text{ Na} = 2·32 \text{ Na}_2\text{O}$  (see calculation in previous analysis).

Potash is combined with sulphuric anhydride.

Now 94 parts  $\text{K}_2\text{O}$  require 80  $\text{SO}_3$ ,

therefore  $0·19 \times \frac{80}{94} = 0·16 \text{ SO}_3$  required,

and  $0·19 \text{ K}_2\text{O} + 0·16 \text{ SO}_3 = 0·35 \text{ K}_2\text{SO}_4$ .

We now combine the remainder of the sulphuric anhydride with soda.

80 parts of  $\text{SO}_3$  require 62 parts of  $\text{Na}_2\text{O}$ .

Then  $0.61$  (amount of  $\text{SO}_3$  remaining)  $\times \frac{62}{80} = 0.47$   $\text{Na}_2\text{O}$  required, and  $0.47 \text{ Na}_2\text{O} + 0.61 \text{ SO}_3 = 1.08 \text{ Na}_2\text{SO}_4$ .

We now deal with the remainder of our soda.

$2.32$  ( $\text{Na}_2\text{O}$  combined with  $\text{Cl}$ )  $+ 0.47$  ( $\text{Na}_2\text{O}$  combined with  $\text{SO}_3$ )  $= 2.79$  parts of soda,  
and  $6.29 - 2.79 = 3.50$  remaining soda.

Remainder combined as carbonate of soda.

62 parts  $\text{Na}_2\text{O}$  require 44 parts  $\text{CO}_2$ .

Then  $3.50$  (remaining  $\text{Na}_2\text{O}$ )  $\times \frac{44}{62} = 2.48$   $\text{CO}_2$  required,  
and  $3.50 \text{ Na}_2\text{O} + 2.48 \text{ CO}_2 = 5.98 \text{ Na}_2\text{CO}_3$ .

Lime is combined as carbonate.

56 parts of  $\text{CaO}$  require 44 parts of  $\text{CO}_2$ .

Then  $5.72$  (amount of  $\text{CaO}$ )  $\times \frac{44}{56} = 4.50$   $\text{CO}_2$ ,  
and  $5.72 + 4.50 = 10.22 \text{ CaCO}_3$ .

Magnesia combined as carbonate.

40 parts of  $\text{MgO}$  require 44 parts of  $\text{CO}_2$ .

Then  $2.41$  (amount of magnesia)  $\times \frac{44}{40} = 2.65$   $\text{CO}_2$ ,  
and  $2.41 \text{ MgO} + 2.65 \text{ CO}_2 = 5.06 \text{ MgCO}_3$ .

*Results :—*

|   | Grains per gallon. |
|---|--------------------|
| Chloride of sodium (NaCl) ...                           | 4·38               |
| Sulphate of soda (Na <sub>2</sub> SO <sub>4</sub> ) ... | 1·08               |
| Sulphate of potash (K <sub>2</sub> SO <sub>4</sub> )... | 0·35               |
| Carbonate of soda (Na <sub>2</sub> CO <sub>3</sub> )    | 5·98               |
| Carbonate of lime (CaCO <sub>3</sub> )...               | 10·22              |
| Carbonate of magnesia (MgCO <sub>3</sub> )              | 5·06               |
| <hr/>   |                    |
| Total saline residue ...                                | 27·07              |

|                               | Grains per gallon. |
|-------------------------------|--------------------|
| Total alkalinity of water ... | 22·0               |
| Alkalinity after boiling ...  | 5·8                |

The *alkalinity used as a check* on results :—

106 Na<sub>2</sub>CO<sub>3</sub> is equal to 100 CaCO<sub>3</sub>,

$$\text{Then } 5·98 \times \frac{100}{106} = 5·64 \text{ as CaCO}_3,$$

84 MgCO<sub>3</sub> equals 100 as CaCO<sub>3</sub>,

$$\text{Then } 5·06 \times \frac{100}{84} = 6·02 \text{ as CaCO}_3,$$

Therefore—

|   | Grains per gallon. |
|---|--------------------|
| Alkalinity due to CaCO <sub>3</sub> ... | 10·22              |
| „ „ MgCO <sub>3</sub> ...               | 6·02               |
| „ „ Na <sub>2</sub> CO <sub>3</sub> ... | 5·64               |
| <hr/>                                   |                    |
|   | 21·88              |

Each of the salts above obtained may also be converted into its equivalent sulphate, and the total so obtained compared with the estimated sulphated residue. In this way we find

|  | Grains per gallon. |
|--|--------------------|
| Chloride of sodium expressed as sulphate ... | 5·25               |
| Sulphate of soda ...                         | 1·08               |
| Sulphate of potash ...                       | 0·35               |
| Carbonate of soda expressed as sulphate ...  | 8·01               |
| Carbonate of lime „ ...                      | 13·90              |
| Carbonate of magnesia „ ...                  | 7·23               |
| <hr/>  |                    |
| Total salts expressed as sulphates...        | 35·82              |

This is within a sufficiently close agreement to that directly determined.

**Indirect Determination of Alkalies.**—If the alkalies are to be determined by difference the method followed is exactly as described in the first example, *i.e.*, the lime and magnesia are calculated to their respective sulphates, and the sum of these two salts subtracted from the total sulphated residue.

The use of factors will be found to considerably shorten the working out of water results, and a table of these is given on p. 289, but as it is most important that the student should fully understand how these are obtained, the working out of the water in the two examples has been given in detail, and the explanation there given how such factors are obtained.

In the present example the lime and magnesia are calculated into their equivalent sulphates by using the factors 2.43 and 4.00 respectively.

Multiplying 5.72 by the factor 2.43 we obtain 13.9, the equivalent of sulphate of lime.

Multiplying 2.41 by the factor 3.00 we obtain 7.23, the equivalent of sulphate of magnesia.

Adding these results together 21.13 is obtained as the total sulphates of lime and magnesia, and, subtracting this from 34.50, we obtain 13.37 as the alkalies, expressed in the form of sulphates.

Multiplying by the factor 4.36 we obtain 5.83 grains per gallon of total alkalies expressed as  $\text{Na}_2\text{O}$ .

Having obtained the alkalies in this way the remaining calculations are worked out as already described, that is to say, the sodium being in excess, the chlorine is first combined with soda, and the further calculations continued as on p. 285.

Table of Factors for Use in Calculating Results of Water Analysis.

| A.                            | B.  | Factor. | C.                              | D.                              | Factor. |
|-------------------------------|---|---------|---------------------------------|---------------------------------|---------|
| CaO                           | CaCO <sub>3</sub>                             | 0·560   | CaO                             | N <sub>2</sub> O <sub>5</sub>   | 0·518   |
| 2MgO                          | Mg <sub>2</sub> P <sub>2</sub> O <sub>7</sub> | 0·362   | Na <sub>2</sub> O               | SO <sub>3</sub>                 | 0·775   |
| 2KCl                          | K <sub>2</sub> PtCl <sub>6</sub>              | 0·307   | Na                              | Cl                              | 0·648   |
| K <sub>2</sub> O              | 2KCl  | 0·631   | Ca                              | Cl <sub>2</sub>                 | 0·563   |
| Na <sub>2</sub> O             | 2NaCl   | 0·530   | K <sub>2</sub> O                | SO <sub>3</sub>                 | 1·175   |
| Na <sub>2</sub> O             | Na <sub>2</sub> SO <sub>4</sub>               | 0·436   | CaCO <sub>3</sub>               | MgCO <sub>3</sub>               | 1·200   |
| Na <sub>2</sub> O             | Na <sub>2</sub> CO <sub>3</sub>               | 0·584   | Na <sub>2</sub> CO <sub>3</sub> | CaCO <sub>3</sub>               | 1·860   |
| 2Na                           | Na <sub>2</sub> O                             | 0·742   | CaCO <sub>3</sub>               | Na <sub>2</sub> CO <sub>3</sub> | 0·943   |
| MgO                           | MgCO <sub>3</sub>                             | 0·475   | SO <sub>3</sub>                 | MgO                             | 2·000   |
| Mg                            | MgO   | 0·600   | SO <sub>3</sub>                 | CaO                             | 1·420   |
| Ca                            | CaO   | 0·714   | SO <sub>3</sub>                 | Na <sub>2</sub> O               | 1·293   |
| SO <sub>3</sub>               | BaSO <sub>4</sub>                             | 0·343   | SO <sub>3</sub>                 | K <sub>2</sub> O                | 0·851   |
| N <sub>2</sub> O <sub>5</sub> | N <sub>2</sub>                                | 3·857   | CO <sub>2</sub>                 | Na <sub>2</sub> O               | 0·713   |
| N <sub>2</sub> O <sub>3</sub> | N <sub>2</sub>                                | 2·710   | Cl <sub>2</sub>                 | Mg                              | 2·958   |
| P <sub>2</sub> O <sub>5</sub> | Mg <sub>2</sub> P <sub>2</sub> O <sub>7</sub> | 0·635   | Cl <sub>2</sub>                 | Ca                              | 1·775   |

To obtain the substances under Column A from those under Column B, multiply the latter by the factor given

$$= \frac{A}{B}.$$

To obtain the required equivalent of substances under Column C for those under Column D, multiply the latter by the factor given =  $\frac{C}{D}$ .

To convert the following salts into their equivalent sulphates, multiply by the factors given—

|                                   |     |      |                   |     |      |
|-----------------------------------|-----|------|-------------------|-----|------|
| NaCl                              | ... | 1·21 | CaCl <sub>2</sub> | ... | 1·23 |
| Na <sub>2</sub> CO <sub>3</sub>   | ... | 1·34 | CaCO <sub>3</sub> | ... | 1·36 |
| Ca(NO <sub>3</sub> ) <sub>2</sub> | ... | 0·83 | MgCO <sub>3</sub> | ... | 1·43 |
| MgCl <sub>2</sub>                 | ... | 1·26 | KCl               | ... | 1·17 |

## CLASSIFICATION OF WATERS AND INTERPRETATION OF ANALYSES.\*

All fresh, or inland, water is derived from rain, which, descending upon the earth's surface, percolates through the various strata, dissolving in its passage various mineral, and probably also organic substances. It is evident that, according to the geological formation through which rain-water passes, the dissolved constituents will vary, and it is often possible to predicate what salts are most likely to be found in water from any particular formation. E. Frankland thus classifies waters in the order of their excellence :—

|            |     |   |   |
|------------|-----|---|---|
| Pure...    | ... | { | 1. Spring water.                                    |
|            |     |   | 2. Deep-well water.                                 |
|            |     |   | 3. Upland surface water.                            |
| Suspicious | ... | { | 4. Rain water.                                      |
|            |     |   | 5. Surface water from cultivated lands.             |
| Impure     | ... | { | 6. River water, to which sewage water gains access. |
|            |     |   | 7. Shallow-well waters.                             |

This classification is, of course, merely a general one, and admits of considerable sub-classification and variation.

There is, however, another consideration, second only in importance to that of purity, namely: the saline character of a water. The same chemist arranges as to hardness or softness water from various sources in the following order :—

- |  |                          |
|--|--------------------------|
| 1. Rain water.                         | 4. Polluted river water. |
| 2. Upland surface water.               | 5. Spring water.         |
| 3. Surface water from cultivated land. | 6. Deep-well water.      |
|  | 7. Shallow-well water.   |

\* See also *The Examination of Waters and Water Supplies*, by J. C. Thresh, 1904, and *The Geology of Water Supply*, Woodward, 1910.



The solid matter in water varies greatly in quantity, ranging from 0.25 to 200 or 250 grains per gallon. The quantity, of course, depends upon the soil and substrata receiving the rainfall, and through which it percolates.

\*1. *Waters from Granitic Metamorphic Trap-rock and Clay Slate.*—The total solids usually very low, not exceeding 6 grains per gallon; they consist of carbonate and chloride of sodium, with very little lime and magnesia. The quantity of organic matter small.

2. *Millstone Grit and Hard Oölite Waters.*—These resemble the waters last described; they are very pure, and contain carbonate and sulphate of lime, magnesia, and traces of iron; the solids seldom exceed 8 grains per gallon.

3. *Soft Sand-rock Waters.*—Waters derived from this source are frequently impure, and contain much chloride, sulphate, and carbonate of sodium, with but little lime and magnesia. The total solids range from 30 to 80 grains per gallon, and the organic matter is also very high. Occasionally these waters are found pure and soft.

4. *Loose Sand and Gravel Waters.*—Composition extremely variable; solids ranging from 4 to 70 grains per gallon; reaction of water often strongly alkaline, and the organic matter somewhat high. A few are very pure, notably those from the greensand, where extreme purity is sometimes found.

5. *Lias Clay Waters.*—Frequently contain very large quantities of mineral matter—as much, even, as 200 grains per gallon.

\* The following classification is from Dr. Parkes' *Practical Hygiene*.

6. *Chalk Waters*.—These, representing a very large and most useful class, are usually of fair, and sometimes of great, purity, and contain from 5 to 25 grains per gallon of carbonate of lime.

7. *Limestone and Magnesian Limestone*.—These resemble in many respects chalk waters, but contain large quantities of magnesia and lime, which exist generally as sulphate. Sometimes they are of great purity.

8. *Senelic Waters*.—Rich in sulphate of lime, and generally very hard, but often of great purity.

9. *Surface and Subsoil Waters*.—These, of course, vary enormously in composition, but are mostly impure, almost invariably so in populated districts.

10. *Marsh and Moor Waters*.—These are often extremely soft, many moor waters containing a solid residue, not exceeding 5 grains per gallon. Marsh waters are very rich in organic matter; but this is of little importance, as it is usually of a vegetable character. Moor waters are very similar, but contain less organic matter, and are, indeed, sometimes very pure. They generally have a peculiar earthy, or peaty, smell, and are of a slightly yellowish tint.

### Free and Albuminoid Ammonia.

Wanklyn divides by the above indications waters into three classes as follows:—

CLASS I.—Waters of extraordinary organic purity: those yielding from 0.00 to 0.05 part of albuminoid ammonia per million.

CLASS II.—Safe waters: those yielding from 0.05 to 0.10 of albuminoid ammonia per million.

CLASS III.—Impure waters: those yielding more than 0·10 part of albuminoid ammonia per million.

The above classification of Wanklyn must not be accepted too rigidly; indeed, Wanklyn expressed further opinions, which may be summarised as follows:—

1. If the free ammonia exceeds 0·08 per million, recent contamination by urine is indicated. In such case a great excess of chlorides may be expected.

2. If the free ammonia and chlorine are present in only small quantities, and if the albuminoid ammonia comes off slowly on distillation, vegetable contamination is indicated.

3. Unless the albuminoid ammonia exceeds 0·05 part per million, a water may be regarded as pure, even although the proportions of free ammonia and chlorine are large. (For instance, in London deep-well waters.)

4. If free ammonia is present in only small amount, the albuminoid ammonia is not to be regarded as a reason for condemning a sample, unless it reaches 0·1 part per million.

5. If, however, albuminoid ammonia reaches 0·1 part per million, the water is suspicious, apart from the absence of free ammonia and chlorine, whilst if the albuminoid ammonia exceeds 0·15 part per million, the sample is to be absolutely condemned.

It is probable that few chemists would now altogether accept the whole of the above propositions, and Wanklyn appeared to regard vegetable contamination as almost as serious as animal pollution, a view which is certainly not generally held. In connection with the results obtained by

this method it is necessary to point out that organic substances yield very different proportions of their ammonia by this process. For instance, asparagine yields the whole of its nitrogen as albuminoid ammonia, albumen about half, uric acid about one-eighth, urea none.

### Oxygen Absorbed.

This test is a most valuable confirmatory one, and from the amount of oxygen absorbed it is possible to obtain very useful information as to the character of a water. It must be remembered that not merely organic matter, but\* iron in the ferrous state, sulphuretted hydrogen, and nitrites absorb oxygen from permanganate of potash. Correction for these may, however, be made by conducting two experiments as described in the analytical section, for the iron, sulphuretted hydrogen, and nitrites absorb oxygen very rapidly, whilst organic matter does so slowly.

Tidy,† to whom is chiefly due the reintroduction of the process originally devised by Forschhammer, proposed the following standards for the valuation of water by this test, expressing the opinion that the oxygen absorbed roughly represents one-tenth of the organic matter present. It is safer, however, not to attempt to convert the indications of the test into actual quantity of organic matter, but to register the amount of oxygen absorbed.

1. Water of great purity, oxygen not to exceed 0·05 part per 100,000.

\* Nitrates also slightly affect the result, but not sufficiently to be of any importance.

† *Journ. Chem. Soc.*, 1879, p. 46.

2. Water of medium purity, not to exceed 0.15 part per 100,000.

3. Water of doubtful purity, 0.21 part per 100,000.

4. Impure water, more than 0.21 per 100,000.

Tidy particularly protests against the drawing of any hard and fast line, and gives the classification as a suggestion merely. The authors themselves adopt standards more severe than either those of Wanklyn or Tidy. With the oxygen test, as with Wanklyn's, different organic matters yield varying results. Tidy particularly claims that the test is specially useful in detecting putrescent organic matter.

The tests above mentioned are the two upon which chemists chiefly rely for the valuation of the organic character of the water. Some chemists, however, adopt Frankland's combustion process, and determine the amount of organic carbon and nitrogen. The process is extremely complicated, and requires very delicate manipulation. It is not necessary here to discuss the merits of the process, for the determination cannot in any case be made by other than a trained chemist.

## Nitrates.

These are often regarded as evidence of former pollution. Nitrogen-containing organic matters when in a water slowly oxidise into nitric acid, which, of course, does not exist in the free, but in the combined state. The presence of nitrates, therefore, indicates that at some previous period a sample has contained organic matter, and in such a case this is strong evidence against a water, because if a sample has been once polluted, there is always danger of a recurrence.

It is quite possible that a water containing organic matter may, during its oxidation, have become so perfectly filtered through strata, that there is no unoxidised organic matter remaining, and the filtration may, at the same time, have completely removed any organisms. Notwithstanding this, such a water is a very doubtful one, even as a drinking supply, whilst for brewing purposes there is much reason to believe that nitrates are themselves objectionable apart from any indication of pollution which they afford. This is due to the fact that nitrates encourage the growth of bacteria, whilst they are not of any value in the nutrition of yeast. The presence of nitrates therefore involves the supply of bacteria food, as distinct from yeast food. Nitrates are less objectionable in hard than in soft waters. It appears as though the presence of a large amount of saline matter counteracts the injurious action of nitrates, and, in the author's experience, an amount of nitrates which may be most objectionable in a soft water, may have very little effect in a hard supply. Any quantity above  $2\frac{1}{2}$  grains per gallon in a soft water, and 4 grains per gallon in a hard water, will certainly in most cases produce difficulty. There are, however, some cases in which a water may contain nitrates, not as the result of previous pollution, but from the natural presence of such salt in the strata from which the water has been drawn. When this occurs, it is usually with hard waters.

### Nitrites.

The absence of nitrites in a water cannot be taken as an indication that such water is organically pure, but when



nitrites are present, the water should be regarded with some suspicion, particularly if it is known to be from a surface supply or from a shallow well. Nitrites are an intermediate stage in the oxidation of ammonia into nitrates, and as such are indicative of the presence of organic matter, but, as pointed out by Franklin many years ago, nitrites when present in a water from a deep-well supply are absolutely without significance, as in such cases they are formed by the reduction of nitrates by means of iron or in some other way. It is well known that nitrates may be present in deep-well supplies which are proved to be organically perfectly pure, and their presence when so found is simply due to the fact that they are a permanent saline constituent of the water taken up from the strata through which the water has percolated. It is conceivable that traces of nitrites may be present as due to the same cause.

### General Saline Constituents.

In judging the value of saline matters in a water, regard must be had to the particular class of beer which it is proposed to produce. For waters of Burton character a suitable proportion of sulphate of lime is about 50 grains per gallon; if the amount exceeds 75 grains per gallon the beers are usually very slow both in coming into condition and in clearing, and for this reason it is frequently found necessary, even in Burton, to dilute some of the very hard waters with a softer supply, when producing light bitter beers which are intended for early consumption. For such beers (those of AK character, for instance) a proportion of about 30 grains per gallon of sulphate of lime is probably



most desirable. The proportion of magnesia in a water is important, because, if present in large excess, it is generally believed to promote a tendency to fretfulness; and, certainly, in the author's experience, results in thin-drinking beers. The amount of sulphate of magnesia should not exceed at the maximum one-third of that of sulphate of lime.

*Chloride of calcium* is not generally found in brewing waters, but is largely added in artificial hardening. It is useful for decomposing both sulphate and carbonate of soda, and itself appears to communicate some fulness, which is lacking with sulphate of lime, yet without giving a coarse flavour, which is commonly attributed to chloride of sodium (common salt). The amount of chloride of calcium generally added varies between 10 and 15 grains per gallon. On account of its deliquescent character, it is usually prepared and used as a saturated solution, which should have a specific gravity of about 1.370. The addition of a pint of such a solution to every 10 barrels of the water adds about 12 grains of chloride of calcium per gallon. Care should be taken that the chloride of calcium does not contain iron.

*Chloride of Sodium (Common Salt).*—This, though found in only small quantities in many waters, exists in very large proportions in the supplies of certain districts, rising as high as 150 grains per gallon in some cases. Beers brewed with such waters are very full, and possess a peculiar character of their own, which, although unsuitable for pale ales, is much esteemed in mild beers. The artificial addition of common salt to brewing waters is very usually

adopted in mild beer brewing.\* A suitable proportion is from 50 to 75 grains per gallon, and the addition of each pound of common salt per 10 barrels communicates 19 grains per gallon of chloride of sodium. The addition of 3 lbs. per 10 barrels would therefore be suitable for waters to be used for mild beers.

In treating waters for stout brewing it is customary either to simply boil the supply, or to boil and afterwards add common salt, much the same proportion being used as for mild beers. *Carbonate of soda* was at one time added to soften waters, but it is now seldom used for this purpose.

Waters containing carbonate of soda—they usually also contain a good deal of *sulphate of soda*—produce good black beers, but, on account of the harsh hop flavour which they extract, are unsuitable for bitter beers. It is possible to decompose both the carbonate and sulphate of soda by the addition of chloride of calcium, but the amount of resulting chlorides is often so large that a really high-class pale ale can seldom be produced from a water so treated. In such cases it is far better to employ a town supply, if such can be obtained, and is pure, and artificially harden it to the required degree. In the authors' experience, an

\* The legality of the treatment of waters in this way was questioned a few years ago, but it was shown that the addition of any moderate amount of salt could not fairly be held to be an adulteration; nor could the frequent contention of the prosecutors be upheld, that the quantity ordinarily used—solely, of course, for the purpose of adding fulness to the beer—was actually employed for the purpose of creating thirst. It was pointed out by Moritz that milk itself contains about 120 grains per gallon of common salt.

artificially hardened town supply is often capable of producing beers of the very highest pale ale character ; and they do not find that beers produced from naturally hard supplies (which are by no means always pure) have any such superiority over properly treated soft but pure waters, as has been so often asserted.

The hardness of a water may be "temporary" or "permanent." Temporary hardness is due to the presence of carbonate of lime and magnesia, which are not themselves soluble in water, but which combine with carbonic acid to form bi-carbonates of these bases, and these salts are soluble. The hardness from such is temporary, as boiling easily decomposes the salts, liberating carbonic acid gas and the insoluble carbonates of lime and magnesia. The addition of lime water will also destroy temporary hardness, the lime combining with the carbonic acid gas and liberating the insoluble carbonate. Permanent hardness is due to the presence of sulphates, chlorides, and nitrates of lime, magnesia, and soda.

The actual reasons for the peculiarities of a beer brewed with a hard water are not yet fully understood. Originally, it was stated that soft waters, being admittedly more generally solvent, dissolved larger quantities of nitrogenous matters from the malt than did hard supplies ; but analysis has disproved this. This theory being no longer tenable, it was suggested that though the quantity dissolved might be the same in both cases, yet the quality differed. So far, however, as our present imperfect methods of separation of the different types of nitrogenous matters enable us to go, there is no such considerable difference in this respect as to

afford a satisfactory explanation of the observed difference between hard and soft waters. It has, however, been shown that worts prepared with water containing large quantities of lime salts give beers containing low proportions of phosphates—the soluble phosphates being precipitated as phosphate of lime—and to this cause some, at least, of the advantages of such waters may be due. Further than this, it is found that the proteins precipitated by the boiling of the wort separate in a more flocculent condition with hard than with soft waters, whilst, in the case of waters containing considerable quantities of carbonate of soda, the precipitated proteins remain suspended in the wort in an extremely finely divided condition, and obstinately refuse to deposit.

If *gypsum* is used for hardening, allowance must be made for the fact that it consists of hydrated sulphate of lime ( $\text{CaSO}_4 + 2\text{H}_2\text{O}$ ), so that it only contains 79·06 per cent. of actual  $\text{CaSO}_4$ , and a corresponding increase must be made in the amount of gypsum to be added. Thus 12·7 parts of gypsum are required to equal 10 parts of  $\text{CaSO}_4$ . Sulphate of lime is practically equally soluble in cold and hot water (its solubility in cold water is actually very slightly greater than in hot), so that the boiling of the water to be hardened does not assist the solution, save by preventing the subsidence of the gypsum. On account of the comparative difficulty of dissolving gypsum, “precipitated sulphate of lime” is now often employed. This has the advantage of being very readily soluble. In calculating the amount to be used, allowance must be made for the large proportion of water contained in the preparation,

which, indeed, is often sent out in the form of a pasty mass. The ready solubility of the sulphate of lime in many semi-liquid hardening fluids offered to brewers is due to the fact that it is generally in the precipitated form, and is therefore dissolved immediately the semi-liquid is added to the bulk of the water.

Sulphate of magnesia is generally added in the form of Epsom salts, which consists of  $\text{MgSO}_4 + 7 \text{H}_2\text{O}$ , and therefore contains 48.78 per cent. of actual  $\text{MgSO}_4$ . Thus, roughly, 20 grammes of Epsom salts are required to equal 10 grammes of  $\text{MgSO}_4$ .

### Analyses of Waters.

In the table of analyses given on the following pages, Nos. 1 and 2 samples are strong Burton waters of fair purity. The high proportion of sulphate of lime and the absence of carbonate of lime, but presence of a considerable amount of carbonate of magnesia will be noted.

No. 3 shows the composition of a pure town supply of average hardness. The carbonates of the alkaline earths approach a total of 20 grains per gallon. In not a few waters the amount of carbonates is greater than this.

No. 4 is a soft town supply, contaminated with vegetable organic matter.

No. 5 is an average analysis of the well water supplies of the Duddingston district (Edinburgh), which, despite the fact that they contain some nitrates, are excellent brewing waters.

No. 6 is the Loch Katrine supply. Very soft and of great organic purity. These being soft waters, exert considerable solvent action on lead.

No. 7 is water drawn from a badly contaminated stream, the defilement being chiefly with vegetable matter.

No. 8 is a deep-well water in the London district. The presence of carbonate and sulphate of soda and of a large amount of free ammonia will be noticed. This water is of great purity, and produces excellent black beers, but is not a pale-ale water, and can hardly be rendered suitable for this purpose, even after treatment.

No. 9 is a remarkable well water from the West of England. The large amount of carbonate and sulphate of soda, the presence of much free ammonia, yet the great purity of the sample, will be observed.

No. 10. This water is remarkable for containing both chloride of sodium and calcium.

No. 11. A peaty water, representative of many such, especially in the West of England.

| Analyses of Water from<br>Various Sources.                      | Burton<br>Well Water. | Burton Deep<br>Well Water. | Pure Town<br>Supply. | Thames Water<br>(Vegetable<br>Contamination). |
|---|-----------------------|----------------------------|----------------------|---|
|   | 1                     | 2                          | 3                    | 4   |
|   | GRAINS PER GALLON.    |                            |                      |   |
| Total solid residue, dried at<br>212° F. ... ..                 | 108·92                | 105·80                     | 26·60                | 17·36   |
| Saline residue ... ..   | 92·17                 | 91·05                      | 25·83                | 16·53   |
| Organic and volatile matter and<br>water of crystallisation ... | 16·75                 | 14·75                      | 0·77                 | 0·83  |
| Lime ... ..   | 24·61                 | 26·66                      | 12·31                | 6·82  |
| Magnesia ... ..   | 9·67                  | 9·37                       | 0·55                 | 0·50  |
| Soda ... ..   | 4·95                  | 1·78                       | 0·87                 | 1·23  |
| Potash ... ..   | 0·41                  | 0·08                       | nil                  | 0·29  |
| Sulphuric acid ... ..   | 39·57                 | 44·06                      | 1·58                 | 1·54  |
| Nitric acid ... ..  | 0·27                  | 0·86                       | 0·54                 | nil   |
| Nitrous acid ... ..   | nil                   | nil                        | nil                  | nil   |
| Chlorine ... ..   | 5·67                  | 1·54                       | 1·47                 | 1·26  |
| Poisonous metals ... ..   | nil                   | nil                        | nil                  | nil   |
| Iron ... ..   | trace                 | bare<br>trace              | bare<br>trace        | trace   |
|   | PARTS PER MILLION.    |                            |                      |   |
| Ammonia, free ... ..  | 0·015                 | nil                        | nil                  | nil   |
| „ albuminoid ... ..   | 0·065                 | 0·050                      | 0·020                | 0·160   |
|   | PARTS PER 100,000.    |                            |                      |   |
| Oxygen required to oxidise<br>organic matter ... ..             | 0·079                 | 0·065                      | 0·052                | 0·245   |
| Probable combination of above bases and acids :—                | GRAINS PER GALLON.    |                            |                      |   |
| Chloride of sodium ... ..                                       | 9·35                  | 2·54                       | 1·64                 | 2·18  |
| Chloride of calcium ... ..                                      | —                     | —                          | 0·73                 | —   |
| Sulphate of soda ... ..   | —                     | 1·01                       | —                    | —   |
| Sulphate of potash ... ..                                       | 0·76                  | 0·15                       | —                    | 0·54  |
| Sulphate of lime ... ..   | 59·43                 | 63·65                      | 2·76                 | 2·19  |
| Sulphate of magnesia ... ..                                     | 6·39                  | 8·97                       | —                    | —   |
| Nitrate of lime ... ..  | 0·41                  | 1·33                       | 0·82                 | —   |
| Carbonate of soda ... ..  | —                     | —                          | —                    | —   |
| Carbonate of lime ... ..  | —                     | —                          | 18·73                | 10·57   |
| Carbonate of magnesia ... ..                                    | 15·83                 | 13·40                      | 1·15                 | 1·05  |



| Duddingston<br>(Edinburgh)<br>Supply. | Loch Katrine<br>Water. | Impure Town<br>Supply. | London Deep<br>Well. | West of England<br>Well Supply. | Well near Sea<br>Coast. | Peaty Water.    |
|---------------------------------------|------------------------|------------------------|----------------------|---------------------------------|-------------------------|-----------------|
| 5                                     | 6                      | 7                      | 8                    | 9                               | 10                      | 11              |
| GRAINS PER GALLON.                    |                        |                        |                      |                                 |                         |                 |
| 30·80                                 | 4·76                   | 12·04                  | 45·64                | 77·28                           | 292·88                  | 4·76            |
| 28·50                                 | 4·48                   | 10·64                  | 42·56                | 75·60                           | 258·80                  | 3·80            |
| 2·30                                  | 0·28                   | 1·40                   | 3·08                 | 1·68                            | 34·08                   | 0·96            |
| 9·72                                  | 0·55                   | 3·75                   | 3·14                 | 3·43                            | 28·38                   | 0·42            |
| 1·31                                  | 0·25                   | 0·50                   | 0·70                 | 2·12                            | 10·38                   | 0·15            |
| 2·41                                  | 0·95                   | 0·92                   | 17·60                | 27·09                           | 92·54                   | 1·40            |
| 0·32                                  | trace                  | 0·24                   | trace                | trace                           | 3·76                    | nil             |
| 4·22                                  | 0·24                   | 1·63                   | 7·97                 | 8·35                            | 17·19                   | 0·19            |
| 2·54                                  | nil                    | nil                    | nil                  | nil                             | 2·59                    | nil             |
| nil                                   | nil                    | nil                    | nil                  | nil                             | nil                     | nil             |
| 2·10                                  | 1·12                   | 1·05                   | 6·37                 | 17·57                           | 132·30                  | 1·61            |
| nil                                   | nil                    | nil                    | nil                  | nil                             | nil                     | nil             |
| trace                                 | nil                    | trace                  | heavy<br>trace       | trace                           | very<br>heavy<br>trace  | barest<br>trace |
| PARTS PER MILLION.                    |                        |                        |                      |                                 |                         |                 |
| 0·010                                 | 0·012                  | 0·195                  | 0·32                 | 0·630                           | 0·005                   | 0·010           |
| 0·040                                 | 0·010                  | 0·160                  | 0·05                 | 0·020                           | 0·050                   | 0·175           |
| PARTS PER 100,000.                    |                        |                        |                      |                                 |                         |                 |
| 0·047                                 | 0·041                  | 0·153                  | 0·06                 | 0·015                           | 0·085                   | 0·258           |
| GRAINS PER GALLON.                    |                        |                        |                      |                                 |                         |                 |
| 3·46                                  | 1·80                   | 1·73                   | 10·51                | 28·99                           | 174·60                  | 2·65            |
| —                                     | —                      | —                      | —                    | —                               | 41·39                   | —               |
| 1·33                                  | —                      | —                      | 14·14                | 14·87                           | —                       | —               |
| 0·59                                  | —                      | 0·44                   | —                    | —                               | 6·96                    | —               |
| 5·44                                  | 0·41                   | 2·43                   | —                    | —                               | 5·18                    | 0·32            |
| —                                     | —                      | —                      | —                    | —                               | 16·47                   | —               |
| 3·86                                  | —                      | —                      | —                    | —                               | 3·93                    | —               |
| —                                     | —                      | —                      | 10·01                | 20·03                           | —                       | —               |
| 11·07                                 | 0·67                   | 4·93                   | 5·60                 | 6·12                            | —                       | 0·52            |
| 2·75                                  | 0·52                   | 1·05                   | 1·47                 | 4·45                            | 10·27                   | 0·31            |

**Kainit.**

This substance is obtained from mines at Stassfurt, Germany, and is believed to be the deposit from an inland sea. Its composition is most variable, and it is frequently dirty and impure.

|  |     |     |     | Per cent.    |
|--|-----|-----|-----|--------------|
| Sulphate of potash                           | ... | ... | ... | 19·34        |
| Sulphate of magnesia                         | ... | ... | ... | 17·99        |
| Chloride of magnesia                         | ... | ... | ... | 10·88        |
| Chloride of sodium                           | ... | ... | ... | 38·06        |
| Insol. silica, etc.                          | ... | ... | ... | 0·30         |
| Water, moisture and water of crystallisation |     |     |     | 13·43        |
|  |     |     |     | <hr/> 100·00 |

**Gypsum.**

|                          |     |     |     |              |
|--------------------------|-----|-----|-----|--------------|
| Sulphate of lime         | ... | ... | ... | 79·06        |
| Calcic oxide             | ... | ... | ... | 32·54        |
| Sulphuric anhydride      | ... | ... | ... | 46·52        |
| Water of crystallisation | ... | ... | ... | 20·94        |
|                          |     |     |     | <hr/> 100·00 |

**Epsom Salts.**

|                          |     |     |     |              |
|--------------------------|-----|-----|-----|--------------|
| Sulphate of magnesia     | ... | ... | ... | 48·78        |
| Magnesium oxide          | ... | ... | ... | 16·24        |
| Sulphuric anhydride      | ... | ... | ... | 32·54        |
| Water of crystallisation | ... | ... | ... | 51·22        |
|                          |     |     |     | <hr/> 100·00 |

## CHAPTER IX.

## SULPHITES.

## BISULPHITE OF LIME.

THE following are the estimations to be made :—

- |                             |                    |
|-----------------------------|--------------------|
| 1. Specific gravity.        | 6. Lime.           |
| 2. Sulphurous acid (total). | 7. Magnesia.       |
| 3. Sulphurous acid (free).  | 8. Alkalies.       |
| 4. Sulphuric acid.          | 9. Iron.           |
| 5. Chlorine.                | 10. Hyposulphites. |

**Specific Gravity.**

This is taken in the ordinary manner in a specific gravity bottle, the only precaution necessary at this point being to make the determination as rapidly as possible after the sample is drawn, to obviate alteration in weight from evaporation or oxidation of the free sulphurous acid. For the same reason, the sample should be preserved in a well-stoppered bottle, and all measurements made as quickly as possible.

**Total Sulphurous Acid.**

This may be determined in several ways, either gravimetrically or volumetrically. In the former methods, the sulphurous acid, both free and combined, is oxidised into sulphuric acid, and determined by means of baric chloride,

calculating from the weight of precipitated baric sulphate obtained the amount of sulphurous acid.

First, however, we must determine the weight of baric sulphate which may be due to any sulphuric acid existing as such in the bisulphite, and deduct this from the total weight of baric sulphate, calculating the difference into sulphurous acid.

To oxidise the sulphurous acid, either bromine or chlorine is employed. Proceed as follows :—

20 c.c. of the bisulphite is measured into a 200 c.c. flask, and diluted to mark. Now place in a beaker 100 to 150 c.c. of distilled water, and about 20 c.c. of bromine water—made by shaking up bromine with distilled water—and run into this 10 c.c. of the solution of bisulphite (= 1 c.c. of bisulphite). After the addition of the bisulphite, the solution should remain distinctly brown in colour. Should it not be so, some more bromine water must be added until the colour is permanent. A little hydrochloric acid is now added, and the solution boiled until it loses its colour, and the whole of the free bromine is expelled. The sulphurous has now been oxidised into sulphuric acid, the bromine decomposing the elements of water and combining with the hydrogen to form hydrobromic acid, whilst the sulphurous acid seizes the oxygen thus liberated. The sulphuric acid is now determined in the solution in exactly the same manner as in “Water Analysis,” and the sulphurous acid calculated after deducting the  $\text{BaSO}_4$  due to the sulphuric acid naturally present in the sample.

An example will make this clear. 10 c.c. of a 10 per cent. solution was taken (= 1 c.c. bisulphite), oxidised by

means of bromine water, then hydrochloric acid added, and the solution boiled. The weight of the  $\text{BaSO}_4$  obtained from this solution by precipitation with baric chloride was 0.255 gramme, but 1 c.c. of the bisulphite was found to give 0.005 of  $\text{BaSO}_4$  as due to sulphates naturally present, so that  $0.255 - 0.005 = 0.25$   $\text{BaSO}_4$  from sulphurous acid ( $\text{SO}_2$ ) in 1 c.c.

Now, the molecular weight of baric sulphate being 233, and that of sulphurous anhydride 64, it follows that 233 parts of  $\text{BaSO}_4$  correspond to 64 parts  $\text{SO}_2$ , or 1 gramme to 0.2746 gramme, and  $0.25 \times 0.2746 = 0.06865$  gramme  $\text{SO}_2$  in 1 c.c. bisulphite, or 6.86 per 100 c.c.

When employing chlorine as an oxidiser in place of bromine, either a saturated solution of chlorine may be added, or the gas itself is passed directly into the solution from a generating flask containing a mixture of manganic dioxide and strong hydrochloric acid which is gently heated.

### Sulphuric Acid.

100 c.c. of distilled water and 10 c.c. of concentrated hydrochloric acid are placed in a small flask and boiled vigorously for five or six minutes to expel the air in the flask and the dissolved oxygen in the water; 50 c.c. of bisulphite is added and the ebullition continued until the evolved steam no longer smells of sulphurous acid. This occurs in about a quarter of an hour. The contents of the flask are now transferred to a beaker, diluted slightly with distilled water, and baric chloride solution added in slight excess, the resulting precipitate being filtered, well washed with hot water, dried, ignited, and weighed as  $\text{BaSO}_4$ .

*Example :—*

|                            |     |     |     | Grammes. |
|----------------------------|-----|-----|-----|----------|
| Crucible + ash =           | ... | ... | ... | 16·500   |
| Tare of crucible ...       | ... | ... | ... | 16·219   |
| Ash                        | ... | ... | ... | 0·281    |
| Deduct ash of filter paper | ... | ... | ... | 0·001    |
|                            |     |     |     | 0·280    |

Then  $0·280 \times 0·343 = 0·096$   $\text{SO}_3$  in 50 c.c. bisulphite, and  $0·096 \times 2 = 0·192$  gramme per 100 c.c.

### Lime.

20 c.c. of the diluted bisulphite solution (20 c.c. in 200 c.c.) is placed in a beaker (= 2 c.c. bisulphite) with about 150 c.c. of distilled water, and ammoniac oxalate, ammoniac chloride, and ammoniac hydrate successively added, each in slight excess. The liquid is gently warmed and allowed to remain at rest for three hours, then filtered, the precipitate washed with hot water, dried, ignited, re-carbonated, and weighed as calcic carbonate ( $\text{CaCO}_3$ ) in the manner described under "Water Analysis."

*Example :—* 2 c.c. bisulphite treated as above described, gave, after ignition and re-carbonation, the following figures :—

|                      |     |     |     | Grammes. |
|----------------------|-----|-----|-----|----------|
| Crucible + ash =     | ... | ... | ... | 16·302   |
| Tare of crucible ... | ... | ... | ... | 16·217   |
| Ash                  | ... | ... | ... | 0·085    |
| Deduct ash of filter | ... | ... | ... | 0·003    |
| Carbonate of lime    | ... | ... | ... | 0·082    |

Then  $0.082 \times 0.56 = 0.0459$  CaO in 2 c.c. bisulphite,  
and  $0.0459 \times 50 = 2.29$  grammes CaO per 100 c.c.

### Magnesia.

To the filtrate from lime estimation is added ammonic phosphate in slight excess and ammonic hydrate until the liquid smells strongly ammoniacal. It is then well stirred, and set aside for six hours, then filtered, the precipitate washed with dilute ammonia, dried, ignited, and weighed as magnesian pyrophosphate.

*Example :—*

|                           |     |     |     | Grammes. |
|---------------------------|-----|-----|-----|----------|
| Crucible + ash =          | ... | ... | ... | 16.228   |
| Tare of crucible ...      | ... | ... | ... | 16.216   |
| Ash                       | ... | ... | ... | 0.012    |
| Deduct for ash of filter  | ... | ... | ... | 0.003    |
| Pyrophosphate of magnesia | ... | ... | ... | 0.009    |

And  $0.009 \times 0.36 = 0.0032$  magnesia in 2 c.c. bisulphite,  
and  $0.0032 \times 50 = 0.16$  gramme in 100 c.c.

### Alkalies (Soda and Potash).

These are usually present only in traces, but if it is desired to estimate the amount it can be done as follows :—

Measure 50 c.c. of the bisulphite into a porcelain dish and evaporate to dryness on the steam bath. Now add about 25 c.c. of distilled water; stir well, and filter the solution into a small beaker, washing the dish and filter paper once or twice with hot water. The alkalies can now be determined in the solution as described in "Water Analysis."



## Iron.

25 c.c. of the bisulphite is boiled in a flask for five minutes with 50 c.c. of distilled water and about 2 c.c. of strong hydrochloric acid. A little bromine water is now added, and the contents of the flask again boiled until no more bromine fumes are given off. The solution is then cooled, transferred to a 100 c.c. measure flask and diluted to mark, and the iron estimated by means of potassium ferrocyanide solution and standard iron as described under Sugar Analysis, p. 205.

That is to say 5 c.c. of standard iron solution is prepared as there described and afterwards run into a 100 c.c. flask, making up to the mark with distilled water at 60° F. The 100 c.c. of standard solution so prepared contains 0.5 milligramme of iron (Fe).

Two or three different quantities of the standard solution are now taken and each made up to the 50 c.c. in a Nessler tube, the potassium ferrocyanide solution added to both it and the bisulphite solution and comparisons made to obtain a similar depth of colour in each.

For accuracy the comparison must be made on equal volumes, and it is soon ascertained by a little practice what strength of standard iron solution must be made up to compare with the bisulphite solution.

In this laboratory we find useful tubes of standard colours made with copper sulphate solution and a few drops of ferric chloride solution, each tube being of similar colour to known iron standards.

*Example :—*

The iron in 50 c.c. of this dilute bisulphite solution (25 per cent.) was found to equal in colour depth 20 c.c. of the standard diluted iron solution also made up to 50 c.c. The 50 c.c. of bisulphite solution therefore contained 0.1 milligramme of iron (Fe).

Therefore  $0.1 \times 8 = 0.8$  milligramme of Fe in 100 c.c. of the bisulphite, or 0.0008 gramme.

It is, however, usual to express the iron in bisulphite as ferric oxide ( $\text{Fe}_2\text{O}_3$ ). Now 112 parts of iron equal 180 parts of ferric oxide ( $\text{Fe}_2\text{O}_3$ ), or 0.7 gramme = 1 gramme.

Therefore  $0.7 : 1 :: 0.0008 = 0.00114$  gramme  $\text{Fe}_2\text{O}_3$  per 100 c.c. of bisulphite, or 0.8 grains per gallon.

**Chlorine.**

20 c.c. of bisulphite is taken and evaporated to dryness in a porcelain dish over a water-bath. When dry, the residue is moistened with a little distilled water, and re-dried, this treatment being repeated two or three times. The residue is now extracted with boiling distilled water, thrown on a filter, the filtrate and washings received into a beaker, cooled, a few drops of potassic chromate added, and the solution titrated with standard silver nitrate, as in "Water Analysis." As an example, 20 c.c. of bisulphite thus treated required 0.5 c.c. standard silver solution; then, as each cubic centimetre of this corresponds to 0.001 gramme of chlorine,  $0.5 \times 0.001 = 0.0005$  chlorine in the 20 c.c. of bisulphite, or 0.0025 gramme per 100 c.c. This is brought into terms of chloride of sodium by multiplying by the factor 1.65; thus  $0.0025 \times 1.65 = 0.0041$  chloride of sodium per 100 c.c. of bisulphite.

## Hyposulphites (or Thiosulphates).

These bodies cannot exist in any sample which has been prepared with care, and are, indeed, never met with in the products of any firm of repute. They can, in fact, only be present if the bisulphite is merely a by-product—as is sometimes said to be the case—from other chemical manufactures. They may be easily detected by adding to the bisulphite concentrated hydrochloric—or, indeed, any strong mineral—acid, and raising to boiling, when, if hyposulphites are present, a yellow precipitate of sulphur is thrown down. This precipitate must not be confused with one of a white colour sometimes obtained by this treatment; it is distinctly yellow, and cannot be mistaken if once observed. If present, it is unnecessary to estimate its amount, as the sample must be at once unhesitatingly rejected as being wholly unfit for use.

## Combinations.

Having completed the foregoing determinations, it now remains to combine the various acids and bases found. This is carried out in the following manner:—

First take the 0.19 of sulphuric acid ( $\text{SO}_3$ ) and saturate this with its equivalent of lime ( $\text{CaO}$ ). Thus:—

$$80 : 0.19 :: 56 = 0.13 \text{ CaO}$$

required for the  $\text{SO}_3$ , combining with it to form 0.32  $\text{CaSO}_4$  (sulphate of lime) per cent.

Taking next the remainder of our lime 2.16 ( $2.29 - 0.13$ ), we saturate it with sulphurous acid ( $\text{SO}_2$ ), the molecular weight of which is 64,

$$56 : 2.16 :: 64 = 2.47,$$

the  $\text{SO}_2$  combining with 2.16 of  $\text{CaO}$  to form 4.63  $\text{CaSO}_3$  (sulphite of lime).

Having now saturated our lime, we take the magnesia, combining this also with sulphurous acid, the molecular weight of  $\text{MgO}$  being 40, thus :—

$$40 : 0.16 :: 64 = 0.25,$$

$\text{SO}_2$  combining with 0.16  $\text{MgO}$  to form 0.41  $\text{MgSO}_3$  (magnesian sulphite) per cent.

The “free sulphurous acid” is calculated by deducting the sum of that saturated by the lime and magnesia from the total amount found. Then

$$(1) \ 2.47 + 0.25 = 2.72$$

$$(2) \ 6.86 - 2.72 = 4.14 \text{ “free sulphurous acid.”}$$

The chlorine has already been expressed as sodium chloride ( $\text{NaCl}$ ); whilst the iron has also been calculated to ferric oxide, in which form it is stated in the analysis.

The whole analysis thus stands as under :—

Specific gravity at  $60^\circ \text{ F.}$ ,  $1070^\circ$ .

|                             |     | Per cent.  |
|-----------------------------|-----|------------|
| Total sulphurous acid       | ... | 6.86       |
| Free sulphurous acid        | ... | 4.14       |
| Combined sulphurous acid... | ... | 2.72       |
| Calcium sulphite            | ... | 4.63       |
| Magnesium sulphite          | ... | 0.41       |
| Calcium sulphate            | ... | 0.32       |
| Sodium chloride             | ... | 0.004      |
| Ferric oxide...             | ... | 0.0057     |
| Hyposulphites               | ... | <i>nil</i> |

The preceding results are all expressed on 100 c.c. or 100 parts of the bisulphite by volume. Frequently, however, the figures of analysis are required to be stated on 100 parts by weight. This can easily be done by a simple calculation. The specific gravity of the bisulphite being 1070°, the actual weight of 100 c.c. of the liquid is 107 grammes, or 100 parts by volume equal 107 parts by weight; therefore, each figure of the analysis multiplied by 100/107 will give the results expressed in percentages on the latter. For example :—

$$\text{Total SO}_2 = 6.86 \text{ in 100 c.c.}$$

Therefore,  $107 : 100 :: 6.86 = 6.41$  in 100 grammes of bisulphite,

or 100 parts by weight contain 6.41 per cent.

## SOLID SULPHITES.

When analysing dry calcium sulphite, or sodium or potassium sulphites, the same general methods as those above given are adopted, the quantities being used as under :—

### Calcium Sulphite.

Determine lime (CaO) in 0.20 gramme.

„ sulphurous acid (SO<sub>2</sub>) in 0.20 gramme.

„ sulphuric acid (SO<sub>3</sub>) in 1 gramme.

„ iron (Fe) in 5 grammes, boiling up with hydrochloric acid and bromine water, and diluting to 100 c.c.

Some samples of calcium sulphite contain uncombined lime, or carbonate of lime. The presence of either of these is indicated when the acids fail to satisfy the base,

and, if necessary, a special determination of alkalinity or carbonic acid may be made to check such result.

Many samples of calcium sulphite prepared by precipitation contain an equivalent of water of crystallisation, the sulphite existing as  $\text{CaSO}_3 + \text{H}_2\text{O}$ ; in such a case, the analytical results will, of course, not add up to 100 parts even when the moisture has been determined, for the water of crystallisation is not expelled, except at high temperatures.

### Potassium and Sodium Sulphites.

Determine sulphurous and sulphuric acids, and iron. No determination of the bases is generally necessary, but if required it may be made by the method described under "Water Analysis."

## INTERPRETATIONS.

Sulphites of great purity are now prepared by many firms. The sulphite most generally used is still probably bisulphite of lime, though it no longer remains the one preservative that it formerly was. Commercial samples of bisulphite are usually of fair purity. The strength of a sample may be accurately determined by the percentage of its sulphurous acid, free and combined. Bisulphite of lime is usually sent out of a specific gravity of 1065 to 1070, though it is occasionally made for export purposes of 1120 to 1140. If of a specific gravity of 1065, it should contain not less than 6 per cent. of total sulphurous acid, one-half of which will be combined, the remainder existing in the free state. Most samples contain some sulphate of lime, but the amount of this should not exceed 0.2 or 0.3 per

cent. in a carefully prepared sample. Traces of magnesia and chlorides of the alkalies are occasionally present, but exert no deleterious influence. Thiosulphates are objectionable if present, but are now scarcely ever found. Formerly the sulphurous acid, from which sulphites were made, was prepared by the burning of sulphur. In this way a mixture of oxides of sulphur was produced, amongst them occasionally being hyposulphurous acid. Sulphurous acid is now, however, usually prepared either by heating coke which has been moistened with sulphuric acid, or by boiling sulphuric acid in the presence of sulphur. By either process very pure acid is obtained. In the manufacture of bisulphite of lime, the sulphurous acid is conducted into a series of lead-lined tanks, containing water and chalk, lime, or marble. Sulphite, and afterwards bisulphite of lime, is thus produced.

Monosulphite of lime is now employed to a considerable extent. It is practically insoluble in water, and is without odour or taste. Under the influence of the organic acids present in beer, sulphurous acid is gradually liberated, and thus it is claimed by the advocates of this preparation that the sulphurous acid is only present when actually required, and the stench due to sulphuretted hydrogen, which is sometimes noticeable with bisulphite of lime, is avoided. In our experience, however, the question of the production of sulphuretted hydrogen from a sulphite depends chiefly upon the relative cleanness of the beer to which that sulphite is added, and if a beer is not clean the objectionable smell is produced whatever form of sulphite has been employed. Monosulphite of lime is



usually of great purity, though badly made samples sometimes contain carbonate of lime. The sulphites of soda and potash, though often sold as neutral sulphites, are seldom so actually. In the case of the soda salt, there is almost invariably some excess of sulphurous acid present. The theoretical percentage of  $\text{SO}_2$  present in the neutral soda salt is 50·8. In many commercial preparations the percentage of  $\text{SO}_2$  reaches nearly to 60. These sulphites are very prone to decomposition, sulphate of soda being formed. An anhydrous meta-bisulphite of potash exists which possesses the advantage of being perfectly stable, and, if properly prepared, undergoes no change, even after years. It is now much employed for preservative purposes

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## CHAPTER X.

**ORIGINAL GRAVITY OF BEER.\***

AN exhaustive chemical analysis of a beer is seldom necessary, but complete analysis of wort and beer is described in the next chapter. Microscopical examination of the deposit and general examination of the beer before and after forcing is of great importance, and is described in Chapter XIII.

The determination of the original and present gravities is frequently required in order to ascertain the strength of any sample. These are carried out as follows:—

**Original Gravity.**

The original gravity of a beer may be described as the specific gravity of the wort, before fermentation, from which the beer under examination was prepared.

When wort ferments, its gravity is lowered by the decomposition of the fermentable matters, resulting in the formation (as chief products) of alcohol and carbonic acid in nearly equal proportion, the former remaining in the liquid,<sup>†</sup> and, from its low specific gravity, diminishing its specific weight.

If, then, we remove the alcohol, and bring the beer to the same bulk as before, we shall be able to ascertain the specific gravity of the liquid minus alcohol, or, in

\* Investigations have been undertaken by Sir T. E. Thorpe and Dr. H. T. Brown by direction of the Board of Inland Revenue with a view of preparing a new table of Original Gravities, and their report is now under consideration by a committee appointed for the purpose.

† There is always a slight loss of alcohol, which is probably carried off mechanically with the carbonic acid gas evolved.

other words, of the matter still remaining as unfermented extract in the beer. If we also ascertain the gravity of the separated alcohol, we may find how much fermented extract it indicates, since we know that 100 parts of sugar are decomposed into 52 parts of alcohol and 48 parts of carbonic acid, with traces of succinic and other acids. The practical details of the methods of determining original gravity have been carefully investigated by Graham, Hoffmann, and Redwood, and from their observations the following table has been drawn up, and adopted by the Excise authorities :—

Table by Graham, Hoffmann, and Redwood, showing the Strength of Wort Corresponding to Spirit Indication.

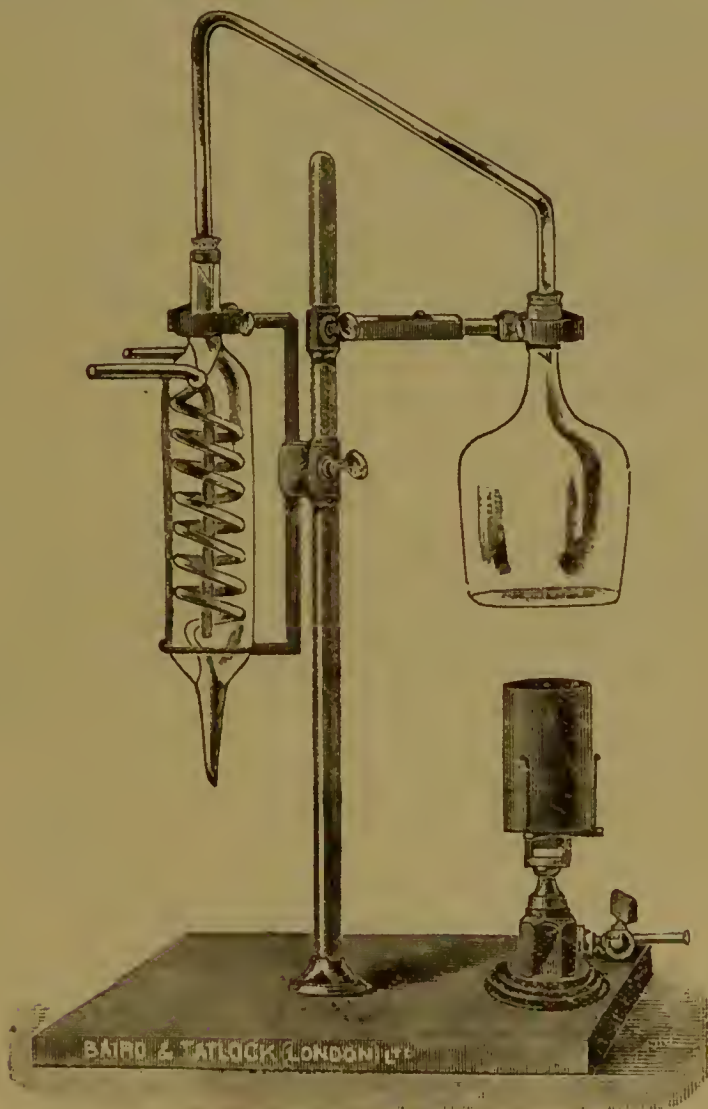
| Degrees<br>of Spirit<br>Indication. | 0·0  | 0·1  | 0·2  | 0·3  | 0·4  | 0·5  | 0·6  | 0·7  | 0·8  | 0·9  |
|-------------------------------------|------|------|------|------|------|------|------|------|------|------|
| 0                                   | 0·0  | 0·3  | 0·6  | 0·9  | 1·2  | 1·5  | 1·8  | 2·1  | 2·4  | 2·7  |
| 1                                   | 3·0  | 3·3  | 3·7  | 4·1  | 4·4  | 4·8  | 5·1  | 5·5  | 5·9  | 6·2  |
| 2                                   | 6·6  | 7·0  | 7·4  | 7·8  | 8·2  | 8·6  | 9·0  | 9·4  | 9·8  | 10·2 |
| 3                                   | 10·7 | 11·1 | 11·5 | 12·0 | 12·4 | 12·9 | 13·3 | 13·8 | 14·2 | 14·7 |
| 4                                   | 15·1 | 15·5 | 16·0 | 16·4 | 16·8 | 17·3 | 17·7 | 18·2 | 18·6 | 19·1 |
| 5                                   | 19·5 | 19·9 | 20·4 | 20·9 | 21·3 | 21·8 | 22·2 | 22·7 | 23·1 | 23·6 |
| 6                                   | 24·1 | 24·6 | 25·0 | 25·5 | 26·0 | 26·4 | 26·9 | 27·4 | 27·8 | 28·3 |
| 7                                   | 28·8 | 29·2 | 29·7 | 30·2 | 30·7 | 31·2 | 31·7 | 32·2 | 32·7 | 33·2 |
| 8                                   | 33·7 | 34·3 | 34·8 | 35·4 | 35·9 | 36·5 | 37·0 | 37·5 | 38·0 | 38·6 |
| 9                                   | 39·1 | 39·7 | 40·2 | 40·7 | 41·2 | 41·7 | 42·2 | 42·7 | 43·2 | 43·7 |
| 10                                  | 44·2 | 44·7 | 45·1 | 45·6 | 46·0 | 46·5 | 47·0 | 47·5 | 48·0 | 48·5 |
| 11                                  | 49·0 | 49·6 | 50·1 | 50·6 | 51·2 | 51·7 | 52·2 | 52·7 | 53·3 | 53·8 |
| 12                                  | 54·3 | 54·9 | 55·4 | 55·9 | 56·4 | 56·9 | 57·4 | 57·9 | 58·4 | 59·0 |
| 13                                  | 59·4 | 60·0 | 60·5 | 61·1 | 61·6 | 62·2 | 62·7 | 63·3 | 63·8 | 64·3 |
| 14                                  | 64·8 | 65·4 | 65·9 | 66·5 | 67·1 | 67·6 | 68·2 | 68·7 | 69·3 | 69·9 |
| 15                                  | 70·5 |      |      |      |      |      |      |      |      |      |

There are several methods of determining original gravity of beer, but the only one it is necessary to describe is that known as the "distillation method," which is that adopted by the Excise.

The details of the process are as follows:—200 c.c. of beer is taken at a temperature of 60° F., the flask being accurately filled to the mark. This is easily accomplished if the beer is flat, but if it is in much condition, exact measurement is not possible. In such cases it is best to filter the beer through a dry funnel and filter paper, collecting in a dry beaker so as to avoid any dilution. By this means the gas is to a great extent expelled, and the liquid may then be measured with accuracy. The expulsion of carbonic acid is not only an advantage on this account, but afterwards much annoyance and trouble will be spared by the absence of frothing during distillation. Having measured 200 c.c. of the beer, it is transferred to a capacious flask, which is attached to a still or condensing apparatus. There are several forms of these; one of the best known is "Liebig's" still, which consists of a glass tube of, say,  $1\frac{1}{2}$  inches diameter, surrounded by another tube or jacket of glass, copper, or tin, through which a constant stream of water is made to flow. Another, the "spiral" still, is an apparatus consisting of a spiral coil of glass tube surrounded with cold water. This form of condenser is to be preferred, and has now almost entirely replaced the older forms, on account of the more perfect and rapid condensation which it secures.

Having fitted the flask containing the beer to the still, a flame is placed underneath, and distillation is commenced. Collect in the measuring flask originally used,

after it has been carefully washed out with distilled water. The boiling is continued until some two-thirds of the liquid has come over, when the flame is removed.



DISTILLING APPARATUS.

The "spirit" has now been all expelled from the beer in the boiling flask, and is contained in the distillate. To estimate its amount and value in degrees, the contents of the 200 c.c. flask are now made up with distilled water to the original bulk at 60° F., thoroughly mixed, and the specific gravity taken in a specific gravity bottle, care being observed that the temperature of the spirit is exactly 60° F. when this is done. The actual weight compared with 1000 is then deducted from that number, and this result is termed the "spirit indication." From this indication, the amount of decomposed or fermented extract is calculated. Thus, if the specific gravity of the spirit had been found to be 991.1, then the spirit indication would be—

$$1000 - 991.1 = 8.9.$$

To bring this to the amount of fermented sugar extract equalled by such figure, we have merely to refer to the table furnished by the Excise. Thus (by table)  $8.9 = 38.6$  degrees of gravity lost during fermentation.

Now to ascertain the extract still remaining unattenuated in the beer, the contents of the boiling flask must be made up to their original bulk, when the specific gravity of the liquid taken at 60° F. gives directly that of the unfermented matter. To do this, the extract is washed into the measuring flask (200 c.c.) and filled up to the mark in the neck at 60° F., the specific gravity being then taken. Assuming that this was found to be 1020.2, the original gravity of the beer would be—

|                     |     |     |     |              |
|---------------------|-----|-----|-----|--------------|
| Degrees fermented   | ... | ... | ... | 38.6         |
| Degrees unfermented | ... | ... | ... | 1020.2       |
|                     |     |     |     | <hr/> 1058.8 |

which, expressed in "lbs. per barrel gravity," would equal  
 $58.8 \times 0.36 = 21.2$  lbs.

In determining the original gravity of old and acid beers, a correction for any excess of acidity must be made, as described later under "Acidity."\*

### Present Gravity.

This is easily taken by filling the specific gravity bottle with the beer at 60° F. (previously filtered, if necessary, in order to flatten it), weighing, and calculating to terms of 1000.

It is always advisable to take the present gravity of a beer when determining its original gravity, as it serves as a check upon the results, in this way:—The present gravity of beer is produced by the solution weight of saccharine and other substances, diminished, more or less, by the alcohol present. Now as we have determined the specific gravity of the alcohol present, we have only to deduct the "spirit indication" from the weight of the extract, when we obtain the present gravity of the beer. Thus:—

|         |     |     |   |        |
|---------|-----|-----|---|--------|
| Extract | ... | ... | = | 1020.2 |
|---------|-----|-----|---|--------|

|                   |     |   |     |
|-------------------|-----|---|-----|
| Spirit indication | ... | = | 8.9 |
|-------------------|-----|---|-----|

|                 |     |   |        |    |           |
|-----------------|-----|---|--------|----|-----------|
| Present gravity | ... | = | 1011.3 | or | 4.1 lbs., |
|-----------------|-----|---|--------|----|-----------|

while a direct experiment as above described gave 1011.5, thus corroborating the distillation results.†

\* The original gravity of beer, as determined by analysis and calculated by table, is always below that of the unfermented wort. The difference usually amounts to about two degrees.

† The difference between calculated and observed present gravities may amount to 0.2° or even 0.3° in black beers.



## Dry Extract.

This is ascertained from the specific gravity of the unfermented extract obtained in the determination of the original gravity. The excess weight over 1000, divided by the factor 3·86, will give the dry extract.

Thus :—

$$\begin{array}{r} 1020\cdot2 \text{ specific gravity of residue.} \\ 1000\cdot0 \\ \hline 20\cdot2 \div 3\cdot86 = 5\cdot23. \end{array}$$

This gives the dry extract in grammes per 100 c.c. of beer. It may also be determined by the evaporation of a definite bulk of beer—say 25 c.c.—to dryness in a tared platinum dish, first over the steam, and afterwards in the water bath, till the weight is constant; it is then calculated into terms of 100 c.c. This figure is useful in order that the constituents in the unfermented extract may be expressed on the dry solids, thus establishing a means of comparison between the solid matter in beers of different gravities and degrees of attenuation.

## Alcohol.

This is ascertained from the specific gravity of the spirit by means of the alcohol table on p. 327.

The specific gravity having been found to be 991·1, this by table is equal to 11·09 per cent. of proof spirit,\* or 5·07 per cent. of absolute alcohol by weight, and it is perhaps more convenient to express results in the latter form.

\* Proof spirit contains 49·24 per cent. of absolute alcohol by weight.

| Specific Gravity<br>at 60° F. | Absolute Alcohol<br>per cent.<br>by weight. | Per cent. of<br>Proof Spirit. | Specific Gravity<br>at 60° F. | Absolute Alcohol<br>per cent.<br>by weight. | Per cent. of<br>Proof Spirit. | Specific Gravity<br>at 60° F. | Absolute Alcohol<br>per cent.<br>by weight. | Per cent. of<br>Proof Spirit. |
|-------------------------------|---|-------------------------------|-------------------------------|---|-------------------------------|-------------------------------|---|-------------------------------|
| 995·0                         | 2·74  | 6·02                          | 992·8                         | 4·02  | 8·81                          | 990·6                         | 5·39  | 11·79                         |
| 994·9                         | 2·79  | 6·13                          | 992·7                         | 4·08  | 8·94                          | 990·5                         | 5·45  | 11·92                         |
| 994·8                         | 2·85  | 6·26                          | 992·6                         | 4·14  | 9·07                          | 990·4                         | 5·51  | 12·05                         |
| 994·7                         | 2·91  | 6·39                          | 992·5                         | 4·20  | 9·20                          | 990·3                         | 5·58  | 12·20                         |
| 994·6                         | 2·97  | 6·52                          | 992·4                         | 4·27  | 9·36                          | 990·2                         | 5·64  | 12·33                         |
| 994·5                         | 3·02  | 6·63                          | 992·3                         | 4·33  | 9·49                          | 990·1                         | 5·70  | 12·46                         |
| 994·4                         | 3·08  | 6·76                          | 992·2                         | 4·39  | 9·62                          | 990·0                         | 5·77  | 12·61                         |
| 994·3                         | 3·14  | 6·89                          | 992·1                         | 4·45  | 9·75                          |                               |   |                               |
| 994·2                         | 3·20  | 7·02                          | 992·0                         | 4·51  | 9·88                          | 989·9                         | 5·83  | 12·74                         |
| 994·1                         | 3·26  | 7·16                          |                               |   |                               | 989·8                         | 5·88  | 12·87                         |
| 994·0                         | 3·32  | 7·29                          | 991·9                         | 4·57  | 10·01                         | 989·7                         | 5·96  | 13·02                         |
|                               |   |                               | 991·8                         | 4·64  | 10·16                         | 989·6                         | 6·02  | 13·15                         |
| 993·9                         | 3·37  | 7·40                          | 991·7                         | 4·70  | 10·29                         | 989·5                         | 6·09  | 13·30                         |
| 993·8                         | 3·43  | 7·53                          | 991·6                         | 4·76  | 10·42                         | 989·4                         | 6·15  | 13·43                         |
| 993·7                         | 3·49  | 7·66                          | 991·5                         | 4·82  | 10·55                         | 989·3                         | 6·22  | 13·59                         |
| 993·6                         | 3·55  | 7·79                          | 991·4                         | 4·88  | 10·68                         | 989·2                         | 6·29  | 13·74                         |
| 993·5                         | 3·61  | 7·92                          | 991·3                         | 4·94  | 10·81                         | 989·1                         | 6·35  | 13·87                         |
| 993·4                         | 3·67  | 8·05                          | 991·2                         | 5·01  | 10·96                         | 989·0                         | 6·42  | 14·02                         |
| 993·3                         | 3·73  | 8·18                          | 991·1                         | 5·07  | 11·09                         |                               |   |                               |
| 993·2                         | 3·78  | 8·29                          | 991·0                         | 5·13  | 11·22                         | 988·9                         | 6·49  | 14·17                         |
| 993·1                         | 3·84  | 8·42                          |                               |   |                               | 988·8                         | 6·55  | 14·30                         |
| 993·0                         | 3·90  | 8·55                          | 990·9                         | 5·20  | 11·38                         | 988·7                         | 6·62  | 14·45                         |
|                               |   |                               | 990·8                         | 5·26  | 11·51                         | 988·6                         | 6·69  | 14·60                         |
| 992·9                         | 3·96  | 8·68                          | 990·7                         | 5·32  | 11·64                         | 988·5                         | 6·75  | 14·73                         |

## Acidity.

The acidity in beer is chiefly due to the presence of organic acids and acid phosphates, and newly brewed beer can contain scarcely any acetic acid ; yet acidity in original gravity determination is expressed in terms of percentages of acetic acid. This mode of stating results was originally adopted by Graham, Hoffmann, and Redwood, in their

experiments on the determination of the original gravity of beer, it being then assumed that the acid normal to beer was acetic. The question of acidity of beer has a direct bearing upon the determination of original gravities, because if acid has been formed, it must have been at the expense of the alcohol or sugar, which will therefore be indicated too low by the original gravity experiment. The authorities quoted have made allowance for such contingency, and have published a table by which any excess of normal acidity may be calculated back to "spirit indication" and allowed for in that table.

Table for Ascertaining the Value of the Acetic Acid.

| Excess per cent.<br>of Acetic Acid<br>in Beer. | Corresponding Degrees of "Spirit Indication." |      |      |      |      |      |      |      |      |      |
|--|---|------|------|------|------|------|------|------|------|------|
|  | 0·00  | 0·01 | 0·02 | 0·03 | 0·04 | 0·05 | 0·06 | 0·07 | 0·08 | 0·09 |
| 0·0  | —   | 0·02 | 0·04 | 0·06 | 0·07 | 0·08 | 0·09 | 0·11 | 0·12 | 0·13 |
| 0·1  | 0·14  | 0·15 | 0·17 | 0·18 | 0·19 | 0·21 | 0·22 | 0·23 | 0·24 | 0·26 |
| 0·2  | 0·27  | 0·28 | 0·29 | 0·31 | 0·32 | 0·33 | 0·34 | 0·35 | 0·37 | 0·38 |
| 0·3  | 0·39  | 0·40 | 0·42 | 0·43 | 0·44 | 0·46 | 0·47 | 0·48 | 0·49 | 0·51 |
| 0·4  | 0·52  | 0·53 | 0·55 | 0·56 | 0·57 | 0·59 | 0·60 | 0·61 | 0·62 | 0·64 |
| 0·5  | 0·65  | 0·66 | 0·67 | 0·69 | 0·70 | 0·71 | 0·72 | 0·73 | 0·75 | 0·76 |
| 0·6  | 0·77  | 0·78 | 0·80 | 0·81 | 0·82 | 0·84 | 0·85 | 0·86 | 0·87 | 0·89 |
| 0·7  | 0·90  | 0·91 | 0·93 | 0·94 | 0·95 | 0·97 | 0·98 | 0·99 | 1·00 | 1·02 |
| 0·8  | 1·03  | 1·04 | 1·05 | 1·07 | 1·08 | 1·09 | 1·10 | 1·11 | 1·13 | 1·14 |
| 0·9  | 1·15  | 1·16 | 1·18 | 1·19 | 1·21 | 1·22 | 1·23 | 1·25 | 1·26 | 1·28 |
| 1·0  | 1·29  | 1·31 | 1·33 | 1·35 | 1·36 | 1·37 | 1·38 | 1·40 | 1·41 | 1·42 |

They have assumed that the normal acidity of a beer is 0·10 per cent. expressed as acetic acid, and any larger amount than that is allowed for by the method mentioned.

Let us suppose that we have found by experiment that the beer upon which we were operating contained an acidity equal to 0.17 per cent. expressed as acetic acid. The Excise have allowed in their calculation 0.1 per cent.; therefore there is a remainder, to be corrected for, of 0.07 per cent. ( $0.17 - 0.1 = 0.07$ ). Now, referring to the table, we find this corresponds to  $0.1^{\circ}$  of "spirit indication," and 0.1 added to our previous "spirit indication" = 9.0 ( $8.9 + 0.1$ ), which by table gives 39.1, and this would raise the original gravity before mentioned from 1058.8 to 1059.3.

In actually determining the acidity of beer, the process is as follows:—100 c.c. of beer is measured into a beaker or porcelain dish, and titrated with decinormal alkali, adding the standard solution a few cubic centimetres, and then tenths of a cubic centimetre, at a time, ascertaining the exact point of neutrality by means of delicate newly prepared litmus paper. Let us suppose that we require 16 c.c. of our decinormal alkali to effect this. The calculation is then as follows:—The standard solution being decinormal, each cubic centimetre of it will correspond to 0.006 gramme of acetic acid, or 0.009 gramme of lactic acid; and as for original gravity purposes the total acidity is reckoned as acetic, the calculation is therefore  $16 \times 0.006 = 0.096$  per cent., so no correction would be necessary in an original gravity determination.

In expressing the acidity of a beer, in ordinary analytical results, it is usual to do so in terms of lactic acid, the decinormal acid used being multiplied by the factor 0.009 ( $16 \times 0.009 = 0.144$  per cent. as lactic acid); but whichever way expressed, we have not gained in this manner any real

knowledge of the character of the acid reacting substances present.

We may divide these into volatile and non-volatile. For this purpose we take a further quantity of the beer (200 c.c.) and distil over as much as it is safe to take off. The flask is then detached, 200 c.c. of water added, and the distillation continued. This addition of water and distillation is twice repeated, when the mixed distillates will contain the whole of the volatile acid. This, or an aliquot portion, is titrated, and the result expressed as acetic acid. The non-volatile acid is expressed as lactic acid.

A convenient instrument known as the *Alcoholometer* has been devised for determining the original gravity of a beer, and is much used where it is required to determine this in a number of samples at a time, and where extreme accuracy is not required.

The method of working depends upon the fact that the boiling point of water is lowered in proportion to the amount of alcohol present. In this way a scale attached to the mercury tube of the instrument is marked in degrees which, when added to the present gravity of the beer, gives the original gravity. The boiling point will of course vary with the pressure, so that before making estimations the instrument must be corrected for zero by first boiling with water alone. The flame used for heating the beer must not vary in size from that used in obtaining the zero reading, and each instrument should be carefully standardised against determinations made by the ordinary distillation process, and the correction for the particular instrument, often two or three degrees, obtained and used with subsequent estimations.

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## CHAPTER XI.

**COMPLETE ANALYSIS OF MALT WORT  
AND BEER.**

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**MALT WORT.**

THE analysis of malt wort is by no means easy. Formerly, it was considered sufficient to determine the reducing sugars and express these as maltose, then to boil a portion of the wort with dilute acid in order to convert the dextrin into dextrose, the maltose being simultaneously hydrated into the same body. The reducing sugars then found were, after deducting the amount of sugar corresponding to the maltose, calculated into the equivalent of dextrin by multiplying the difference by 0·9.

This method is incorrect, and for several reasons. First, the reducing sugars in the wort consist not of maltose only, but likewise of dextrose and lævulose, whilst cane sugar is also present. Second, after boiling with acid, if we carry the process far enough to hydrate all the dextrin, we shall have decomposed some of the reducing sugars, have changed the cane sugar into invert, and have transformed some of the albuminoids into reducing bodies. It is thus clear that the estimation as above sketched is not even approximately correct, and for any satisfactory analysis of wort to be made, it is necessary to take into account and make a correction for these other bodies contained in the malt. The method of analysis here given is based on that originally



proposed by Heron, in which, by an examination both of the wort and of the cold-water extract of the malt (the latter being subjected to the same conditions as the wort), it is possible to accurately determine the amount of the starch products (maltose and dextrin) in a wort. The process may be carried out as follows:—

### Cold Extract.

Prepare a 10 per cent. cold extract of the malt, as described under “Matters Soluble in Cold Water” in “Malt Analysis.”

Take 200 c.c. of the clear, filtered liquid and boil five to ten minutes. Cool, make up to the original bulk, mix, and filter through a dry paper into a dry beaker.

The following determinations are then made on this 10 per cent. cold extract of the malt:—

**1. Ready-formed Soluble Carbohydrates.**—Determine as described under “Malt Analysis.” If these bodies have already been determined, the figures can simply be brought forward, and used for the purpose of the wort analysis.

In the following example, the figures obtained for “ready-formed soluble carbohydrates” under “Malt Analysis” will be so brought forward, and used for the purpose of the calculations.

*Example:—*

“Ready formed soluble carbohydrates” found on

100 parts of malt = 16.48.

Then 10 grammes malt contain 1.648 *grammes*.



**2. Cupric Oxide Reducing Power.**—This is determined as described under “Sugar Analysis,”\* using 30 c.c. of Fehling’s solution, 150 c.c. of distilled water, and 20 c.c. of the 10 per cent. cold malt extract.

*Example :—*

CuO obtained = 0.284 gramme.

Then  $0.284 \times 5 = 1.42$  on 100 c.c. of the 10 per cent. malt solution.

**3. Optical Activity.**—Take some of the clear liquid, and fill a 200 millimetre tube at 60° F. Place in polarimeter, and take reading. (For details of working of polarimeter, see p. 400.) Divide the reading by 2, to give the reading in 100 millimetre tube.

*Example :—*

Optical activity in 200 mm. tube = 1.22°

$$= \frac{1.22}{2} \text{ or } 0.61^\circ \text{ in 100 mm. tube.}$$

## Hot Extract.

The preceding estimations having been made in the cold-water extract, we now examine the wort from the hot-water mash. Before proceeding with the analysis, take 300 c.c. of the wort, and boil for 20 minutes. Cool to 60° F., make up to 300 c.c. again, and filter through a dry paper into a dry vessel.

\* P. 194.

Make the following determinations :—

1. Specific gravity.
2. Cupric oxide reducing power.
3. Optical activity.
4. Nitrogenous bodies.
5. Mineral matter.

**1. Specific gravity** degrees are calculated to percentage of dry solids in the wort by dividing the excess weight over 1000 by the factor 3·86.

*Example :—*

$$\text{Specific gravity} = 1029\cdot0.$$

Then  $29\cdot0 \div 3\cdot86 = 7\cdot50$  per cent. dry solids in wort.

**2. Copper Reducing Power.**—Make up Fehling's solution with 50 c.c. water and 15 c.c. each cupric sulphate and alkaline tartrate solution, as before described. Boil, and add 20 c.c. of a 10 per cent. solution of wort. Proceed precisely as before stated, and calculate cupric oxide to percentage on wort.

*Example :—*

CuO obtained 0·133 gramme = amount in 20 c.c. of a 10 per cent. solution of wort, or 2 c.c. of wort.

Then  $0\cdot133 \times 50 = 6\cdot65$  grammes in 100 c.c. wort.

**3. Optical Activity.**—50 c.c. of the wort—which must be quite clear—is diluted with distilled water to 100 c.c., and the optical activity taken in a 100 millimetre tube.

If, however, the colour of the wort is high, it is prefer-

able to treat it with lead acetate or nitrate, which will clarify the solution, and also remove some of the colour.

To do this, take 50 c.c. of the wort and place it in a 100 c.c. flask, add 10 c.c. of a 10 per cent. solution of plumbic acetate, make up to the 100 c.c. mark, mix thoroughly, and filter through a dry paper into a dry beaker. This will give a 50 per cent. solution of the wort. Take the reading in a 100 millimetre tube.

*Example:—*

Optical activity in a 100 mm. tube =  $4.28^\circ$ .

Then  $4.28 \times 2 = 8.56^\circ$  on 100 parts of wort.

**4. Nitrogenous Bodies.**—These are determined exactly as described under “Malt Analysis,” 10 c.c. of the wort being taken for the estimation.

*Example:—*

10 c.c. gave 0.034 protein.

Then  $0.034 \times 10 = 0.34$  per cent. on wort.

**5. Mineral Matter.**—50 c.c. of wort is evaporated down and burnt off in a platinum dish, as already described in “Malt Analysis.”

*Example:—*

50 c.c. of wort gave 0.046 ash.

Then  $0.046 \times 2 = 0.092$  per cent. on wort.

We are now in possession of the necessary data for the calculation of our results. Now, if we deduct the figures obtained on the digested cold water extract from the

corresponding figures of the hot wort, we have as a remainder the figures due exclusively to starch products.

|                           | Wort. |   | Cold-water<br>Extract. |   | Due to Starch<br>Conversion<br>Products. |
|---------------------------|-------|---|------------------------|---|--|
| Cupric oxide reduction... | 6.65  | — | 1.42                   | = | 5.23                                     |
| Optical activity ...      | 8.56° | — | 0.61°                  | = | 7.95°                                    |

Now each gramme of cupric oxide equals 0.7435 gramme of maltose, and we arrive at the amount of maltose, therefore, by multiplying the cupric oxide obtained by that figure.

Then 5.23 (CuO due to starch products) multiplied by 0.7435 equals 3.89 maltose in wort, expressed in percentage on wort.

Further, 100 grammes of maltose has a rotatory power, when observed in a 100 millimetre tube, of  $135.9^\circ [\alpha]_{D^{3.86}}$ . Thus, 1 gramme equals 1.359. Now, the amount of maltose found is 3.89. So 3.89 multiplied by 1.359 equals 5.28 angle due to maltose. Deduct this from the total angle of starch products, and we have left the angle due to dextrin. The rotatory angle of 100 parts of dextrin is  $194.4^\circ$ , or 1 part equals  $1.944^\circ$ ; therefore the remaining rotatory angle divided by 1.944 gives the amount of dextrin expressed in percentage upon the wort.

Then :—

Total angle  $7.95 - 5.28 = 2.67$  due to dextrin,  
and  $2.67 \div 1.944 = 1.37$  dextrin in 100 parts of wort.

The results may now be stated as under :—

100 parts of the wort contain—

|   |     |        |
|---|-----|--------|
| Maltose (due to starch conversion)  | ... | 3·89   |
| Dextrin (due to starch conversion)  | ... | 1·37   |
| “ Ready-formed soluble carbohydrates ”                                      |     | 1·65   |
| Albumen ... ..  |     | 0·34   |
| Mineral matter ... ..   |     | 0·09   |
| Other bodies (colouring matter, acid, etc.)<br>and error on analysis ... .. |     | 0·16   |
|   |     | <hr/>  |
| Total dry solids ... ..   |     | 7·50   |
| Water (by difference) ... ..  |     | 92·50  |
|   |     | <hr/>  |
|   |     | 100·00 |
|   |     | <hr/>  |

It is, however, preferable to express the composition of a wort in percentage on its dry extract, as this permits of a comparison of the composition of worts, irrespective of their gravities. Now, as 100 parts of wort have been found to contain 7·50 parts of solids, each constituent may be brought to percentages on the dry extract of the wort by a simple calculation.

*Example :—*

The above constituent parts of the wort multiplied by  $\frac{100}{7\cdot50}$  will give the percentage composition of the dry solid extract of the wort, as follows :—

|   |     |     |     |     |                   |
|---|-----|-----|-----|-----|-------------------|
| Maltose                                     | ... | ... | ... | ... | 51.9              |
| Dextrin                                     | ... | ... | ... | ... | 18.3              |
| "Ready-formed soluble carbohydrates"...     |     |     |     |     | 22.0              |
| Albumen                                     | ... | ... | ... | ... | 4.5               |
| Mineral matter                              | ... | ... | ... | ... | 1.2               |
| Other bodies (colouring matter, acid, etc.) |     |     |     |     |                   |
| and error on analysis                       | ... | ... | ... | ... | 2.1               |
|   |     |     |     |     | <hr/> 100.0 <hr/> |

The optical activity of the wort should also be calculated and expressed on the dry solid extract, as it is a useful figure for guidance in the brewing operation.

*Example :—*

Optical activity of wort =  $8.57^{\circ}$ .

Then  $8.57 \times 100 \div 7.50 = 114.2^{\circ}$ .

## Determination of Malto-Dextrins.

In the foregoing method of analysis, we have left out of account one most important fact, namely, that the products of the hydrolysis of starch by diastase include not merely maltose and dextrin, but malto-dextrins also. The importance of these latter bodies is certainly very great, and the analysis of a malt wort which ignores their presence is manifestly unsatisfactory. Moritz and Morris describe a method for the determination of malto-dextrins, basing the process on the complete fermentability of free maltose by primary yeast. Any reducing sugar found after such fermentation is therefore due—subject to a small correction

for other reducing bodies—to combined maltose, existing as malto-dextrin; whilst the dextrin so combined is determined by hydrolysing it into maltose by means of cold water extract of malt, and then determining the increase in reducing sugars. This method is probably the best at present available, but it is open to some objections, which the authors no doubt fully recognise. It is common knowledge that the degree of attenuation of a wort is dependent on the type of yeast employed, the temperature, and the absence or presence of aëration. Now, as with these altering conditions we can obtain different degrees of attenuation, one of two things must be admitted: either with some yeasts we do not always ferment the whole of our maltose during primary fermentation, or under certain conditions primary yeast can and does ferment certain of the malto-dextrins.

If this last proposition be true—and there seems to be strong evidence that it is—how are we to control our fermentations so as to exactly decompose the free maltose, yet avoid loss of malto-dextrin? It may be said that one type of yeast must always be employed. This, however, presents practical difficulties, and, as a rule, the fermentation must be conducted with the ordinary commercial yeast of a brewer. Despite, however, the defects of the process, it certainly gives us very useful information, and it is therefore described substantially as proposed by Moritz and Morris.\* In order to carry it out, we have to make three further experiments: (a) the increase in reducing power of the wort after digestion with cold water extract of malt to

\* *Text-Book of the Science of Brewing.*



hydrolyse the dextrin in the malto-dextrin into maltose, the increase in maltose being calculated to dextrin by multiplying by 0.95 ; (b) the reducing power of the wort after fermentation with yeast ; and (c) the reducing power of the wort after fermentation with the yeast in the presence of cold water extract of malt.

The first experiment gives the amount of combined dextrin, the second that of the combined maltose and other small quantities of reducing bodies, whilst the third gives us the amount of these latter bodies alone. From these results we can calculate the amount of malto-dextrin present in the wort and its type.

In order to carry out the determination, proceed as follows :—

First prepare diastase solution for degradation of malto-dextrins as under :—

200 grammes of finely ground malt (one fairly high in diastase should be chosen) is stirred with 500 c.c. of distilled water, allowed to stand overnight in a cool place, and filtered. The bright filtrate is used for the subsequent experiments. The determination of the reducing power of this is made as follows :—

10 c.c. of the liquid is diluted to 100 c.c., digested one hour at 130° F., then cooled, and the reducing power determined in 10 c.c. (1 c.c. of cold water extract).

$\text{CuO}$  obtained = 0.051 gramme.

This correction must be made where this liquid is used.\*

\* Moritz and Morris suggest that the digestion be prolonged for 48 hours in the presence of  $\frac{1}{2}$  per cent. of chloroform, and they find

The malt wort under examination is found to have a specific gravity of 1029.0. The excess weight over 1000 (29.0) divided by 3.86 gives 7.50 per cent. of dry solids.

*Determination of Combined Dextrin.*

(a) Reducing power of wort :—

20 c.c. of the wort is taken, and diluted to 100 c.c., 10 c.c. of this solution (2 c.c. of wort) is taken, and a gravimetric estimation of sugar made precisely as described under "Wort Analysis."

$\text{CuO obtained} = 0.133 \text{ gramme.}$

(If a complete analysis of wort is being made, this figure will have already been determined.)

(b) Reducing power after digestion with cold water extract (diastase) :—

20 c.c. of wort is now taken, 2.5 c.c. of the diastase solution added, and the whole maintained in a water bath at 130° F. for one hour. The liquid is now cooled and diluted to 100 c.c., and the reducing power determined in 10 c.c.

$\text{CuO obtained} = 0.161 \text{ gramme.}$

As the cold malt extract used contains some reducing bodies, the amount of CuO due to these must be deducted before proceeding with the calculation. Now, as 1 c.c. of the cold extract gave 0.051 CuO, a deduction of 0.013

that such an extract is unchanged for about a fortnight. This obviates the necessity of determining the correction in each experiment, if a number are made.

due to 0.25 c.c. must be made from the total CuO obtained.

|                                      | Gramme. |
|--------------------------------------|---------|
| CuO total... ..                      | 0.161   |
| Deduction for cold water extract ... | 0.013   |
|                                      | <hr/>   |
|                                      | 0.148   |
|                                      | <hr/>   |

Now the CuO due to the maltose and reducing bodies naturally present in the wort amounted to 0.133 gramme.

Therefore  $0.148 - 0.133 = 0.015$  CuO due to maltose from degradation of combined dextrin in 2 c.c. of wort.

and  $0.015 \times 50 = 0.75$  CuO in 100 c.c.,

and  $0.75 \times 0.706^* = 0.529$  combined dextrin in 100 c.c. of wort, or 7.50 of dry solids.

Then  $0.529 \times \frac{100}{7.50} = 7.05$  per cent. of combined dextrin on dry solids.

### *Determination of Combined Maltose.*

(c) Reducing power of wort after removal of free maltose by fermentation :—

50 c.c. of wort is taken, and placed in a small flask, 0.25 gramme of pressed yeast is added, the mouth of flask loosely plugged with cotton wool, the whole placed on forcing tray at about 80° F., and allowed to ferment. The fermentation should be complete in about 48 hours.

\* The factor 0.706 is obtained by multiplying that for maltose (0.7435) by 0.95, for 1 part of maltose is produced from 0.95 part of dextrin.

It should not be allowed to remain much longer if all action has ceased, as the wort may become acid or mouldy, and this may spoil the estimation. The wort is now diluted to 100 c.c. (a small quantity of kieselguhr or alumina being added to assist clarification), then filtered bright through a filter paper, and the reducing power determined in 25 c.c.

CuO obtained = 0.132 gramme.

(d) Reducing power of wort after removal of free maltose and maltose in malto-dextrins (degraded with diastase) by fermentation :—

50 c.c. of wort is fermented as above, but with the addition of 0.25 c.c. of the diastase solution (to degrade the malto-dextrins, and render them fermentable). On completion of the fermentation, a little kieselguhr or alumina is added, and the whole made up to 100 c.c., then filtered, and 25 c.c. of bright filtrate used for determination of reducing power.

CuO obtained = 0.088 gramme.

This is due to reducing bodies contained in wort, other than maltose, and must be deducted from the amount found in (c).

So  $0.132 - 0.088 = 0.044$  CuO due to combined maltose.

Now the 25 c.c. of liquid we have used contains 12.5 of original wort. If, therefore, we multiply by 8 we have the CuO due to maltose per cent. on wort.

$0.044 \times 8 = 0.352$  CuO in 100 c.c. wort,

and  $0.352 \times 0.7435 = 0.262$  combined maltose.

This calculated on dry solids gives :—

$$0.262 \times \frac{100}{7.50} = 3.49.$$

The results are therefore (expressed on dry solids of wort) :—

|                               |     |     |              |
|-------------------------------|-----|-----|--------------|
| Maltose in malto-dextrins ... | ... | ... | 3.49         |
| Dextrin in malto-dextrins ... | ... | ... | 7.05         |
| Total malto-dextrins ...      | ... | ... | <u>10.54</u> |

and the type of malto-dextrin is M. 1, D. 2.

The maltose and dextrin are then shown to be present in the wort in the following condition :—

|                           |     |     |     |       |         |
|---------------------------|-----|-----|-----|-------|---------|
| Free maltose              | ... | ... | ... | 49.71 | } 53.20 |
| Maltose, in malto-dextrin | ... | ... | ... | 3.49  |         |
| Free dextrin              | ... | ... | ... | 9.78  | } 16.83 |
| Dextrin, in malto-dextrin | ... | ... | ... | 7.05  |         |

It must be admitted that the determination of malto-dextrin in wort as thus described involves a good deal of work (the copper determinations should certainly always be made in duplicate), yet it is at present the only method we possess, and in practice becomes less complicated than it may read.

## ANALYSIS OF COPPER WORT.

In dealing with a wort from the copper, etc., a fairly accurate idea of its composition may be obtained by making an analysis as described under "wort from hot water mash," and also an analysis of the cold water extract of the malt from which the brew was made, calculating the correc-

tions due to these bodies by means of a factor to be obtained as follows :—

An ordinary hot water mash of the malt (10 per cent.) is made, and the dry solids in the wort determined.\* Now ascertain the percentage of dry solids in the copper wort, and a simple calculation will give us the factor by which to multiply the results of cold water extract in order that they shall correspond to a hot water mash of a similar strength to that from which our copper wort was obtained.

An example will make this clear :—

Sp. gr. of copper wort ... 1061·8 = 16·01 dry solids.

Sp. gr. laboratory mash... 1029·0 = 7·50 „

Then  $16·01 \div 7·50 = 2·13$ , factor by which to multiply cold water corrections in order to make them correspond with a wort of the same percentage of dry solids as our copper wort (viz., 16·01).

The factor having been ascertained, the calculation is as follows :—

The copper wort gave—

CuO obtained per 100 c.c. wort ... 14·70 grammes.

Optical activity of wort ... 18·92°

The cold water mash (10 per cent. malt) gave—

CuO obtained per 100 c.c. solution 1·42 grammes.

Optical activity of solution ... 0·61°

Ready - formed soluble carbo -

hydrates per 100 c.c. ... 1·64 grammes.

\* Of course, if the extract which the malt yields on a large scale is accurately known it will not be necessary to determine the extract in the laboratory, but the percentage of dry matter which a 10 per cent. solution of the malt would give may be calculated.

These corrected to correspond with the copper wort give—

|                             |     |     |     |                                 |
|-----------------------------|-----|-----|-----|---------------------------------|
| CuO ...                     | ... | ... | ... | $1.42 \times 2.28 = 3.23$       |
| Optical activity            | ... | ... | ... | $0.61 \times 2.28 = 1.39^\circ$ |
| Ready-formed soluble carbo- |     |     |     |                                 |
| hydrates ...                | ... | ... | ... | $1.64 \times 2.28 = 3.74$       |

giving figures as follows due to the maltose and dextrin in the wort :—

$$\begin{aligned}\text{CuO } 14.70 - 3.23 &= 11.47 \\ \text{Angle } 18.92^\circ - 1.39^\circ &= 17.53^\circ\end{aligned}$$

From these and other figures of analysis of the wort, the composition of the dry solids in the sample can be calculated exactly as described under "Wort Analysis."

Thus  $11.47 \times 0.7435 = 8.52$  per cent. maltose on wort,  
and  $8.52 \times 1.380 = 11.75^\circ$  angle due to maltose.

Then  $17.53 - 11.75 = 5.78^\circ$  ,, dextrin,  
and  $5.78 \div 1.944 = 2.97$  per cent. of dextrin on the  
wort.

Therefore the wort contains :—

|                                       |     |     |     |     | Per cent.<br>expressed on wort. |
|---------------------------------------|-----|-----|-----|-----|---------------------------------|
| Maltose                               | ... | ... | ... | ... | 8.52                            |
| Dextrin                               | ... | ... | ... | ... | 2.97                            |
| Ready-formed soluble carbohydrates... |     |     |     |     | 3.74                            |

Or expressed on the dry solids in the wort :—

|                                       |     |     |     |     | Per cent. |
|---------------------------------------|-----|-----|-----|-----|-----------|
| Maltose                               | ... | ... | ... | ... | 53.2      |
| Dextrin                               | ... | ... | ... | ... | 18.5      |
| Ready-formed soluble carbohydrates... |     |     |     |     | 23.3      |



The proportion of malto-dextrins, albumen, mineral matter, etc., may be determined as previously described.

## ANALYSIS OF BEER.

This, as before mentioned, is seldom required, and is not an easy matter to carry out.

The mere determination of the copper-reducing and optically active bodies, and their expression as maltose and dextrin, gives but little information. In order that the analysis shall be of any value, it is necessary to ascertain the condition in which the maltose and dextrin exist in the beer, and the proportion and type of the malto-dextrins which are present. The unfermentable residue of beer also contains a small proportion of copper-reducing and optically active bodies, other than maltose and dextrin, and, therefore, in making an analysis, these must be corrected for, before an accurate determination of the maltose and dextrin can be made.

The most satisfactory and complete method of analysis which we have at present is that drawn up by Morris.\*

In this process the low type malto-dextrins are calculated from the copper reducing power and the optical activity before and after fermentation induced by the active yeast after removal of the alcohol, the results being expressed in terms of maltose. The higher type malto-dextrins and the stable dextrin are determined in much the same manner as in "Malt Wort Analysis," the necessary corrections due to other bodies being made. Fermentation in the presence of

\* *Journ. Inst. Brew.*, vol. 1, p. 125.

cold water extract of malt will remove the whole of the malto-dextrins and the stable dextrin, and the copper reducing power and optical activity of the residue after such fermentation is therefore due to bodies other than maltose, dextrin, and malto-dextrin. The determinations are carried out as follows :—

### **Copper Reducing Power.**

Determine this in 5 c.c. of the beer, or in smaller quantity if necessary.

### **Fermentable Malto-Dextrins.**

Take 300 c.c. of beer, place in boiling flask, and evaporate to half its bulk to expel alcohol, then cool and make up to original volume. Take 100 c.c. of this, add 0.5 gramme of yeast, and place on the forcing-tray. After fermentation is completed, cool, add a little aluminic hydrate, filter, and determine the reducing power in 10 c.c. of the liquid.

### **Normal Malto-Dextrins.**

Take 100 c.c. of alcohol-free beer, add 5 c.c. of cold water malt extract, and digest at about 130° F. for an hour ; then boil, cool, and dilute to 200 c.c. Determine the optical activity and the reducing power, the latter in 5 c.c. Correction must be made for the malt extract, 50 c.c. of which may be taken, raised to boiling point, cooled, and brought to original bulk. Determine reducing power in 2 c.c., also optical activity. The necessary correction must be deducted from the figures obtained as above.

### Reducing and Optically Active Substances, other than Maltose, Dextrin, and Malto-Dextrins.

In order to ascertain these, it is necessary to ferment in the presence of cold water extract of malt. For this purpose take 50 c.c. of alcohol-free beer, and ferment as before, previously adding 2.5 c.c. of cold water malt extract. Determine reducing power and opticity after fermentation, and after diluting to 100 c.c. A correction must be made for the non-fermentable and optically active substances present in the malt extract used, so that a fermentation of this must be conducted beside the other.

The above determinations having been made give us data from which we may calculate—

- (a) The readily-fermentable malto-dextrins.
- (b) The normal malto-dextrins.
- (c) The free dextrin.
- (d) The unfermentable residue.

Morris thus describes the calculation:—The low type malto-dextrins are obtained by deducting the copper oxide after fermentation from the original reducing power, and calculating the difference into maltose. The combined maltose is obtained by deducting the reducing power after fermentation with cold water malt extract from that after fermentation alone. The combined dextrin is calculated from the increase in reducing power after degradation with malt extract. The free dextrin is found from the opticity after degradation, less that due to the total maltose and the unfermentable residue, and the unfermentable residue is obtained from the reducing power after fermentation with

cold water malt extract, the proper corrections for the malt extract itself being applied where necessary.

The following determinations may also be made :—

### Optical Activity of the Beer.

To determine this fill a 100 or 200-millimetre tube with bright beer, and take a reading in the ordinary way. If the beer is too dark to permit of an accurate reading (this is always the case with black beers), it can be treated with lead acetate or decolorised as follows :—

Prepare a bleaching solution thus :—Take about 100 grammes of dry bleaching powder, triturate in a mortar with about 200 c.c. of distilled water, until the whole is reduced to the consistency of a thin cream, and filter. Now take a 100 c.c. flask, place in it 10 c.c. of the above bright bleaching solution, add 50 c.c. of beer to this, and allow to stand for five minutes. Then dilute to mark (100 c.c.), mix, filter, and polarise. The reading obtained multiplied by two will give the angle on the beer. The result is then calculated on 100 parts of the dry solids in the beer.

#### *Example :—*

Reading in a 100 mm. tube =  $2.6^{\circ}$ .

Then  $2.6^{\circ} \times 2 = 5.2^{\circ}$  on beer.

The dry solids in the beer were found to be 5.23 per cent.

Then  $5.2 \times \frac{100}{5.23} = 99.4 [\alpha]_D.$

To clarify with lead acetate proceed as follows :—Take 100 c.c. of the beer, add 10 c.c. of lead acetate solution, mix, and filter through a dry filter paper into a dry beaker.

Take the reading in the polarimeter in the usual way. The reading obtained in the 100-millimetre tube when multiplied by 1.1 will give the results expressed on the beer.

### Nitrogen.

Determine as in malt analysis, using 10 c.c. of the beer. This is carefully evaporated down nearly to dryness and then treated with strong sulphuric acid and potassium sulphate in the usual way.

### Mineral Matter.

Determine on 100 c.c. of the beer. Evaporate down in a platinum dish and ignite carefully as described for malt extract. Where it is required to estimate the *chlorides* present in the beer, the ash must be ignited at as low a temperature as possible. It is finally dissolved in a little distilled water, filtered, and the chlorine determined as described under water analysis (p. 273).

*Sulphates* may be determined in the ash by dissolving in hydrochloric acid, filtering, and precipitating with barium chloride, as described for the estimation of sulphates under water analysis. It is better to add a small quantity of sodium hydroxide to the beer when evaporating down for the purpose of estimating the sulphates, to avoid loss by interaction with phosphates.

### Salicylic Acid in Beer.

It is occasionally desired to ascertain whether salicylic acid is present in beer. The test may be carried out as

follows :—200 c.c. of the beer is evaporated to about 50 c.c. This is then placed in a small bottle or flask, and shaken vigorously for some minutes with about its own bulk of ether. The ether will extract the salicylic acid from the beer, and on standing should separate as a clear liquid on the top of the solution. If it does not do so, a little more ether should be added. The clear ethereal solution is now carefully poured off into a small flask, and distilled off or evaporated on the water-bath (not, however, directly over the steam). The residue is dissolved in a small quantity of water, and one or two drops of ferric chloride solution added. If salicylic acid is present, a violet coloration is produced.

*Sulphites* are often present in beer and can be estimated by distilling 200 or 300 c.c. of the beer with a little hydrochloric acid into water containing a little bromine water. This is then treated as described in Chapter IX, Sulphites, by boiling off the excess of bromine water with the addition of dilute hydrochloric acid and precipitating the oxidised sulphite as sulphate with barium chloride solution.

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## CHAPTER XII.

## ESTIMATION OF ARSENIC IN BREWING MATERIALS.

It is unnecessary to do more than refer to the circumstances which make it essential for all brewing materials to be tested for arsenic.

It will be remembered that in the latter part of 1900 some serious cases of arsenic poisoning occurred in the Manchester district, which were traced to beer brewed with either glucose or invert sugar containing arsenic. The contamination of the sugar was ultimately traced to the use of sulphuric acid in the manufacture, and as much as 3 grains of arsenic per lb. were found in samples of glucose, 4 grains per lb. in samples of invert, and 2 per cent. of arsenious oxide in the acid from the arsenical pyrites used in its preparation.

Several reports were published by the Royal Commission appointed to enquire into the whole matter, and expert evidence was obtained as to the presence of arsenic in brewing and food materials generally. It was then found that arsenic was much more widely distributed than was generally supposed. It was found in malt, for example, in isolated cases up to 1/6th of a grain per lb., the amount found being higher where coke, particularly gas coke, had been used as fuel, this latter containing sometimes more than a grain of arsenic per lb.



Hops, also, were found to contain considerable quantities of the substance, similarly due to the fuel used, or, possibly, in some cases, to the use of insecticide preparations.

With the extreme care taken in the preparation of all brewing materials, it is now very unusual to find any but minute traces of arsenic in beer, but the standard of purity advised by the Royal Commission is somewhat severe, and periodical examination of beers and brewing materials must be made in the laboratory for the presence of arsenic.

The standards advised by the Royal Commission were that all foods or food materials should contain less than  $1/100$ th of a grain of arsenic expressed as arsenious oxide per lb. of the material, and liquids less than  $1/100$ th of a grain per gallon. It was, however, advised that malt ought to contain less than  $1/250$ th of a grain of arsenic per lb.

These are the standards accepted by the Excise authorities, and care should be taken that these quantities are not exceeded.

Concerning yeast foods, the actual percentage used relative to the finished beer is so small compared with such materials as malt or sugar, that for all practical purposes  $1/50$ th of a grain of arsenic per lb. of yeast food is perfectly safe, and the same standard may be adopted for hops; but whilst these more liberal standards are generally agreed upon by chemists connected with the brewing trade, it must not be forgotten that the actual standard recommended is  $1/100$ th of a grain per lb., and, no doubt, if larger quantities than these were found in the materials mentioned, the Excise authorities might very probably draw the brewer's attention to the fact that it contained an excessive amount

of arsenic, though they probably would not actually condemn such materials.

Coal and, of course, more particularly coke, often contain considerable quantities of arsenic, and it would be unsafe to use any fuel for the drying of malt which contained more than  $1/35$ th of a grain of arsenic per lb.

### Methods of Determination.

Several have been devised, but two only can be recommended as at the same time both accurate and sensitive. These are the electrolytic\* and that known as the Berzelius-Marsh test, this latter having been very considerably improved during the last few years, indeed, to such an extent as to make it a very perfect process for the determination of minute quantities of arsenic.

The former or electrolytic method was worked for some time in this laboratory, but it was found that in certain cases its sensitiveness to minute traces of arsenic was considerably less than with the improved Marsh test, and, further, an electric installation is required, not obtainable in every laboratory.

As must be expected with a process in which extremely minute quantities of a substance are to be detected, very great care has to be taken to purify all the reagents used.

The Marsh test now to be described requires great care and attention to details in manipulation, and for these reasons the method is described in very full detail, but even so it is far from being easy, and reliable results can only be obtained in the hands of an experienced operator.

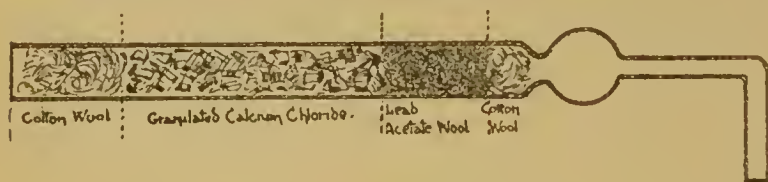
\* For full details of this process, see *Journ. Chem. Soc.*, August, 1903, T. E. Thorpe.

## Apparatus.

A Jena glass flask of about 300 c.c. capacity is used for the generation of hydrogen by means of zinc and hydrochloric acid. Jena or Austrian glass must be used in preference to the ordinary glass, as the latter appears to give off traces of arsenic after having been in use for some time.

The flask is fitted with a two-hole rubber cork, through one hole of which passes a tap funnel of 50 c.c. capacity, and through the other a tube bent at right angles, and enlarged to form a small bulb close up to the flask to retain any liquid which might get carried over mechanically during the evolution of the hydrogen. Next to the bulb the tube is filled with a small plug of cotton wool—about a quarter of an inch in length—followed by about an inch length of lead wool.\*

The remaining part of the tube is loosely packed with granular calcium chloride, and finally plugged with ordinary cotton wool, to keep the calcium chloride in position. No lead wool must be placed after the calcium chloride, and the whole packed tube should measure from 7 to 8 inches.

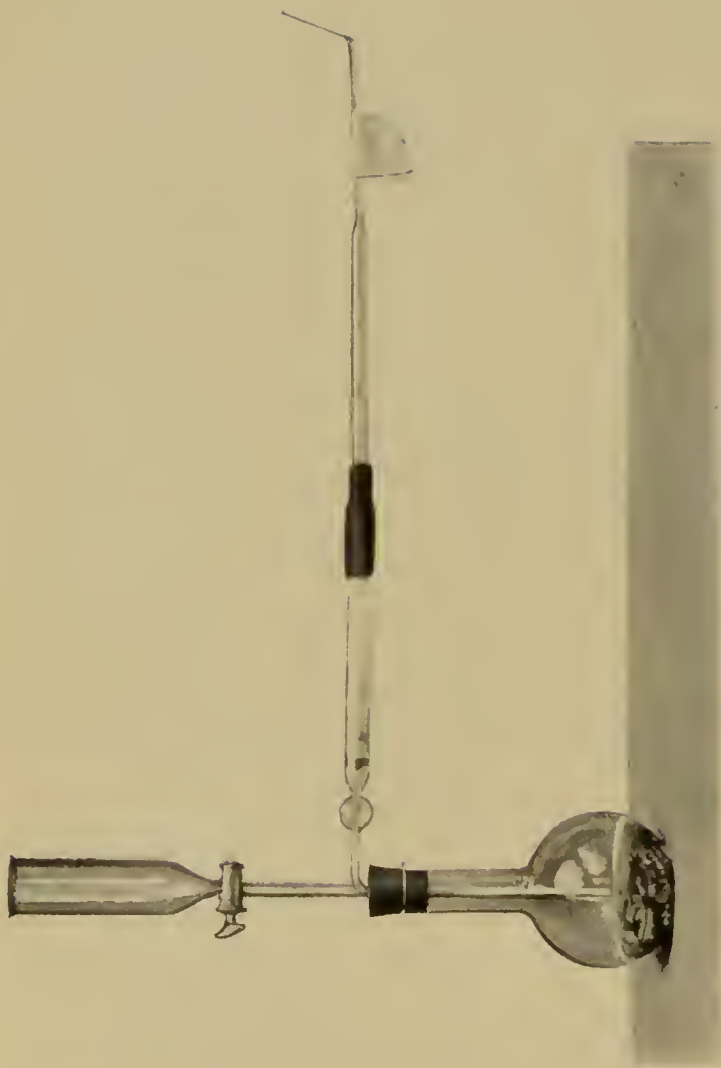


## Glass Tube for Deposit of Arsenic.

This is drawn out just before use from Jena tubing of convenient thickness. An outside diameter of about

\* Prepared by soaking ordinary wool in lead acetate solution and afterwards drying.





ARSENIC APPARATUS—BERZELIUS-MARSH.

10 millimetres and an inside of 8 millimetres will be found satisfactory, and, before drawing this out, it is desirable that a wad of cotton wool should be pushed through the tube to remove any particles of dust which might otherwise cause irregular deposition of the arsenic mirror, and so make the quantitative determination more difficult.

After drawing out the tubing in the blow-pipe flame the narrow part is turned up at right angles, about an inch from the end, and this tube, when cooled, is attached to the calcium chloride tube by a piece of rubber tubing about 2 inches long, which passes over the ends of both, thus binding them firmly together.

The antimony which is present in both rubber stopper and tubing does not appear to interfere with the results in the slightest degree, even after the apparatus has been in use for some years.

The whole apparatus, fitted up in this way, is so arranged that the drawn-out tube passes over a Bunsen burner, which should be of sufficient heating strength to keep the tube at a good red heat.

The "hood" or "chimney" rests on a "star" support, and holds the drawn-out tube in such a position that the edge of the chimney furthest from the flask just touches the shoulder immediately preceding the finely drawn out part, and this latter part of the tube passes over a water trough, placed as close as possible to the chimney, and level with the top of it. The trough is completely filled with water, and a slip of blotting paper about an inch wide bent over the tube with its two free ends dipping into the trough in such a way as to ensure the edge of the paper nearest the burner being at

that point of the tube at which the arsenic is required to deposit—and this is usually found to be about 1 inch distant from the heated portion of the tube along the finely drawn-out part.

In order to concentrate the Bunsen flame uniformly round the glass tubing, a muffle is formed of an ordinary clay scorifier or saucer by inverting this over the tube above the burner—the conditions being such that the muffle extends beyond the edge of the chimney towards the trough, and just overlaps the blotting paper condenser by about one-eighth of an inch.

Satisfactory results depend very largely on the careful carrying out of exact details in the arrangement and fitting up of the apparatus.

The diagrams will explain the various positions of the apparatus, but that facing this page is not quite accurate—the hood and blotting-paper should be nearer together, arranged as described in the text.

## Preparation of Chemicals.

It is first necessary to obtain the zinc and acid free from arsenic.

The chemicals used must of course be perfectly free from arsenic, and at the same time they must be sensitive, that is to say, they must be in such a condition as to give off freely and constantly any arsenic which may be present in the substance under examination.

The difficulty of obtaining zinc free from arsenic, and, at the same time, sufficiently sensitive, was a problem of



{To face p. 358.



ARSENIC APPARATUS—BERZELIUS-MARSH.



considerable difficulty until it was found\* that the addition of a cadmium salt to the zinc greatly increased its sensitiveness. The zinc should contain no excess of iron, as this metal will interfere with the sensitiveness.

1. **Arsenic-free Zinc** may be purchased, but it is safer to prepare it for use as follows :—

Bars of rod zinc are obtained as free as possible from arsenic, and are fused in a graphite crucible in a small box furnace, or they may be equally well melted in an iron ladle. When melted, enough zinc chloride is run on to the surface of the molten zinc to form a layer when fused of about one-eighth of an inch in thickness. This is then allowed to melt, the crucible taken from the furnace, and the mass stirred until it solidifies, thoroughly working in the zinc chloride. The crucible or iron ladle is then reheated, and the zinc again melted and kept in this condition for an hour, the slag being afterwards skimmed off from the surface as completely as possible. It is finally granulated by pouring the molten metal into cold water from a height of about 8 feet. (*Note*.—If the metal is poured into the water from a short distance the zinc does not granulate so well, and forms stud-shaped pieces, which are not desirable.)

2. **Hydrochloric Acid**.—Add sufficient bromine to colour the acid distinctly yellow. Sulphurous acid is then added to just decolourise the bromine, and afterwards a further 20 c.c. of the sulphurous acid in excess.

The hydrochloric acid so treated is then put into a flask attached to an ordinary condensing still and one-fifth of the total volume of the acid is distilled over, the end of the condenser being attached by red rubber tubing to an inverted funnel which is placed in a beaker and covered with water.

When one-fifth has passed over, the funnel and bowl are detached and the remainder of the acid distilled off and collected in the usual way, storing it in either Jena or Austrian glass flasks.

The leading tube of the still and the inner tube of the condenser should be of one piece of Jena tubing bent to the required shape. The condenser is used in the vertical position and all metal fittings, clamps, etc., touching the condenser and which might be attacked by the acid fumes should be protected by binding them over with strips of

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\* A. C. Chapman and H. D. Law, *The Analyst*, 1906, p. 3.

flannel. The end of the tube is connected with the flask by an ordinary cork, a fresh one being used for each distillation.

The acid to be purified should obviously contain as little arsenic as possible. That used in this laboratory is pure redistilled stored in the ordinary pale green Winchesters. White and blue glass seem to give up considerably more arsenic to the acid than does green glass.

### Test.

Having prepared the apparatus and obtained the chemicals themselves satisfactorily free from arsenic, a blank test may now be made to ascertain if the whole apparatus is in working order, and to make sure that no arsenic is deposited in the tube.

Add to the flask a few grammes of granular zinc prepared as described, cover this for a few minutes with dilute sulphuric acid, pour off, and just cover the now clean zinc with hot water. Next run in 5 c.c. of a 20 per cent. solution of cadmium sulphate, and raise the flask contents to the boil. The flask on cooling is then ready for use.

The apparatus is fitted up as already described and about 20 c.c. of the purified hydrochloric acid run in through the tap funnel. The whole is then left until all the air has been driven out, usually two or three minutes, and when this is completed the hydrogen, lit at the jet, will burn at first with a long, narrow, sharp flame, which gradually shortens and finally remains constant and nearly round. With a flame of this shape it may be assumed that the whole of the air is expelled from the apparatus.

The Bunsen burner is now lit and the tube covered with the inverted saucer and the experiment allowed to proceed for 30 minutes, adding a little more arsenic-free hydrochloric acid from time to time through the tap funnel as

this is required to keep the hydrogen flame just burning. The Bunsen flame is then turned out and the tube allowed to cool, when it is carefully cleaned and examined for any deposit of arsenic.

### Standard Tubes.

It is first necessary to have standard tubes containing known quantities of arsenic in order to compare the amounts found in the various materials, and so make the test a quantitative one, and these must be prepared by the operator himself if they are to be of any use.

For this purpose 0.1 gramme of arsenious oxide is dissolved in the smallest amount of water, to which has been added a few very small pieces of stick soda (sodium hydrate). When solution has completely taken place, the whole is diluted with water to about 800 c.c. and then made slightly acid by the addition of arsenic-free hydrochloric acid, finally making up to the mark in a litre flask.

5 c.c. of this are then diluted to 500 and this is used for the standard solution. 10 c.c. of this standard solution tested in the Marsh apparatus as just described will give a deposit equal to 0.01 milligramme or 0.00001 gramme of arsenic.

A series of tubes should be made taking quantities of the standard solution varying from 3 to 10 c.c.; the former amount is equivalent to 0.003 milligramme, and this again represents, on the 10 grammes of malt used as presently to be described, an amount of arsenic equivalent to 1/500 of a grain per lb. of the malt. It is hardly possible and certainly quite unnecessary to obtain tubes to show more delicate indications than this amount.

Standard tubes, if sealed up in a current of hydrogen, should keep unchanged for at least six months.

### **Estimation of Arsenic in Malt.**

40 grammes are weighed out and put into a beaker with about 100 c.c. of boiling tap-water and 10 c.c. of arsenic-free hydrochloric acid, well stirred and allowed to stand for a few moments. The solution is then decanted off through an ordinary funnel into a 200 c.c. graduated flask, taking care to disturb the malt corns as little as possible. This is best carried out by placing a glass rod with a flattened end inside the funnel in such a way as to act as a valve and prevent the grains from getting through into the neck of the funnel.

Further quantities of hot water are then run on to the grain, stirred well, and quickly run off as before, and added to the flask, which is then cooled, made up to the mark, and well mixed.

50 c.c. of this solution (10 grammes of the malt) are then used for the test. In this case the solution is directly added to the Marsh flask without previously charring the organic matter, though it may sometimes be desirable to boil the malt solution for a short time before testing if there is any suspicion of sulphites present in the malt.

The arsenic deposit is afterwards compared with the standard arsenic tubes, and the amount thus estimated.

### **General Preparation of Samples before Testing.**

Except in the case of malt, just described, it is essential to destroy the organic matter in all other brewing materials before they can be extracted in such a condition as to ensure accuracy in testing for arsenic in the Marsh

apparatus. It has been found that unless the charring is very thoroughly carried out, some amount of arsenic appears to be retained in the flask, and the full quantity, therefore, is not deposited on the arsenic mirror in the drawn-out tube.

Further, if sulphites, and sulphur compounds generally, are suspected, they must be oxidised or driven off by boiling with a little hydrochloric acid. Oxidising bodies such as nitric acid, nitrous compounds, bromine, etc., may be present, but whether they are so or not, it is essential in all cases for the solution to be finally treated with a little metabisulphite of potash solution in order to ensure complete reduction of the arsenic present to the arsenious condition. The excess of sulphite should be boiled off before testing the solution in the Marsh apparatus.

**Beer.**—140 c.c. are measured out, placed in a dish with  $2\frac{1}{2}$  c.c. of nitric acid and 15 c.c. of sulphuric acid (1 in 4), afterwards evaporated down and charred as follows :—

After evaporation is complete all the nitrous fumes should be driven off and the whole mass thoroughly blackened; the dish is allowed to cool, and hot water added to the charred mass, and ground with a pestle under water, rubbing down the sides to get all the solid matter down. The solution has again to be evaporated to dryness, and the heating continued over a rose burner until the organic colouring matter is thoroughly discharged, this being complete when the extracted material gives a perfectly colourless solution. This thorough charring is essential, as otherwise arsenic is retained in solution.

The dry mass is finally ground to powder with a pestle,



and covered with about 100 c.c. of water, together with 5 c.c. of arsenic-free hydrochloric acid, and evaporated down over a very small rose burner at such a rate that after about 30 minutes there remains approximately 50 c.c. of the solution.\* This is then filtered into a 100 c.c. graduated flask, and repeatedly washed by decantation with hot water until the filtrate measures 100 c.c. The flask is now cooled, made up to the mark at 60°, and thoroughly mixed; 50 c.c. of this solution are used for the test. Care should be taken that the liquid after the addition of hydrochloric acid is not evaporated down to dryness.

The arsenic in the substance examined must be in the reduced condition, and where solutions are obtained by charring, it is as well just before treatment in the Marsh flask that it should be first transferred to a Jena flask, adding water if necessary, with two or three crystals of potassium metabisulphite, and boiling until all smell of sulphurous acid has disappeared. After cooling back, the solution is then quite ready for directly testing in the Marsh apparatus.

**Sugars and Yeast Foods.**—14 grammes are taken, dissolved, and washed into a porcelain dish; 2.5 c.c. of pure nitric acid, together with 15 c.c. of sulphuric acid (one in four), are added, and the liquid is then treated exactly as in the case of beer, just previously described, that is to say, it is evaporated, charred, and finally made up to 100 c.c., 50 c.c. (or 7 grammes) of which are used for the test.

**Malt Culms and Malt Dust.**—These are often a source of arsenic contamination, particularly where the dust collects on the beams and ledges of the kiln.

28 grammes are extracted by mashing up with 170 c.c.

\* This time is necessary for complete extraction, but evaporation should not decrease the volume much below 50 c.c.

of hot water and 30 c.c. of dilute sulphuric acid (one in four). The liquid is then strained through a funnel having a small plug of cotton wool at the bottom, and the culms are thoroughly pressed until 100 c.c. of the filtrate are obtained = 14 grammes of culms. The 100 c.c. are evaporated in a dish with the addition of  $2\frac{1}{2}$  c.c. of nitric acid, and when dried down the whole is charred, dissolved, and finally made up to 100 c.c. as in the case of beer, and the determination made upon 50 c.c. of the solution, equivalent to 7 grammes of culm or dust.

**Hops.**—20 grammes are taken and mashed up with 35 c.c. of dilute sulphuric acid (one in four), together with 165 c.c. of hot water, the whole kept on a water bath in a Jena beaker covered with a clock glass for about 20 minutes. The mass is then thrown out upon a large porcelain funnel containing a plate perforated with small holes (a Buchner funnel), and the hops are squeezed by means of a glass plate fitted with a knob and which just fits the funnel. By this means about 125 c.c. of liquid can be recovered from the mash.

100 c.c. of this is placed in a dish, 2.5 c.c. of nitric acid added, and the liquid evaporated down, charred, and finally made up to 100 c.c., exactly as in the case of beer; 50 c.c. represents, therefore, 5 grammes of hops, and is used for making the test. It is necessary to char very thoroughly after the second evaporation, as otherwise a coloured solution is obtained.

**Coal and Coke.**—Great care must be taken that the coal or coke is carefully sampled down to manageable

limits. When this has been thoroughly carried out, the resulting small sample is ground into a powder of moderate fineness and thoroughly mixed; 4 grammes are placed in a platinum dish and 5 grammes of lime shaken over it so as to completely cover the coal. The lime is then just moistened with water, and the whole thoroughly mixed into a mud with the help of a glass rod. If the coal does not get thoroughly wetted it is apt to collect on the surface and not mix properly with the lime.

Sufficient water is then added to the paste to completely cover it, and the dish put on the water bath until completely dry, then broken up with a glass rod, thoroughly mixed, and the dish placed over a burner and carefully ignited until all the coal is burnt off.

The powder is now shaken out into a beaker, rinsed with water and about 25 c.c. of hydrochloric acid added, and shaken round for a minute or so, washing in once or twice with fresh portions of the acid, until the whole of the lime is completely dissolved from the dish and collected in the beaker.

This is then raised to the boil, and the boiling continued for about five minutes, then cooled, made up to 100 c.c., and 50 c.c., representing 2 grammes, used for the test.

**Black Malt.**—Extracted as in the case of ordinary malt, but instead of hydrochloric acid use 5 c.c. of sulphuric acid. The solution is then charred in a similar way to that described under beer, after a further addition of 2.5 c.c. of nitric acid.

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## CHAPTER XIII.

# MICROSCOPICAL WORK.

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1. Description of the Microscope.
2. Examination of Yeasts.
3. Examination of Beers.
4. Bacteriological Examination of Water.

It is not proposed to give any detailed account of the microscope and its application to brewing; but this branch of the brewer's work in the laboratory is very important, and some few notes are therefore given under each of the above sections. A number of excellent books have been published dealing with the subject, and to these the student should refer.\*

## I. DESCRIPTION OF THE MICROSCOPE.

### Mechanical Arrangements.

For brewers' purposes a monocular microscope is both most suitable and least costly. The length of tube adopted in Continental instruments is 6 inches, in English 10 inches,

\* *The Microscope in the Brewery and Malt-house*, Matthews and Lott; *Manual of Alcoholic Fermentation*, C. G. Matthews; *Practical Studies on Fermentation*, Hansen; *The Micro-organisms of Fermentation*, Jorgensen; *Studies on Fermentation*, Pasteur.

and the Continental and English objectives are corrected for these lengths of tube. Many English microscopes are now, however, made with a 6-inch tube and a draw-tube, to permit its extension to 10 inches, so that they can be used with either make of objective. The instrument must be provided with both coarse and fine adjustment, and a mechanical stage is an advantage, though somewhat liable to get out of order unless carefully used. Most forms of microscope are provided with an Iris diaphragm, consisting of plates of metal, by a very ingenious arrangement of which the size of the aperture may be regulated at will. Fitted to this sub-stage arrangement is a condenser, by means of which light is collected and concentrated. In working the microscope, the light must be reduced by the use of the diaphragm, for, if too strong, the conformation of the object under examination is not clearly seen, and the eye soon becomes tired.

### **Eyepieces.**

It is a common mistake to use too powerful an eyepiece. It must be remembered that the eyepiece merely magnifies the image, and if that magnification is carried beyond a certain point, which depends upon the quality of the objective, no improvement in clearness or increase of definition is secured, but the reverse. It is far better to work with a fairly high-power objective, and a low-power eyepiece. For most purposes a B eyepiece is sufficiently powerful. The powers of Continental eyepieces are designated by numerals. When immersion lenses are used, relatively high-power eyepieces may be employed.

## Objectives.

Those most suitable for examination of yeast, beer deposit, etc., are  $\frac{1}{8}$  and  $\frac{1}{10}$ -inch objectives. Objectives are usually designated by their focal distance from the object, but in most cases the actual focal distance is considerably less than that implied by the figures. Thus, a  $\frac{1}{4}$ -inch objective has generally to be screwed down to less than a quarter of an inch from the object under examination before it is in focus. In Continental lenses the power is indicated by letters. The following table shows the magnifying power of the various combinations on an English microscope :—

Objectives—6-inch Tube.

| Eyepieces.     | No. 1. | No. 2. | No. 3. | No. 4. | No. 5. | } Dia-<br>meters. |
|----------------|--------|--------|--------|--------|--------|-------------------|
| 1-inch         | 25     | 35.5   | 47     | 60     | 77     |                   |
| $\frac{2}{3}$  | 37     | 50     | 70     | 90     | 115    |                   |
| $\frac{1}{2}$  | 60     | 85     | 115    | 145    | 185    |                   |
| $\frac{1}{4}$  | 80     | 105    | 150    | 180    | 240    |                   |
| $\frac{1}{10}$ | 112    | 168    | 224    | 280    | 335    |                   |
| $\frac{1}{8}$  | 175    | 240    | 325    | 420    | 540    |                   |
| $\frac{1}{6}$  | 198    | 292    | 386    | 492    | 625    |                   |
| $\frac{1}{5}$  | 275    | 412    | 550    | 687    | 824    |                   |
| $\frac{1}{3}$  | 385    | 530    | 730    | 925    | 1180   |                   |
| $\frac{1}{2}$  |        |        |        |        |        |                   |

## 10-inch Tube.

|                |     |      |      |      |      |                   |
|----------------|-----|------|------|------|------|-------------------|
| 1-inch         | 45  | 67.5 | 90   | 112  | 140  | } Dia-<br>meters. |
| $\frac{2}{3}$  | 64  | 96   | 128  | 160  | 192  |                   |
| $\frac{1}{2}$  | 107 | 158  | 214  | 267  | 310  |                   |
| $\frac{4}{10}$ | 126 | 189  | 252  | 315  | 370  |                   |
| $\frac{1}{4}$  | 210 | 315  | 420  | 570  | 600  |                   |
| $\frac{1}{8}$  | 256 | 384  | 512  | 640  | 690  |                   |
| $\frac{1}{8}$  | 342 | 513  | 684  | 855  | 900  |                   |
| $\frac{1}{10}$ | 475 | 712  | 950  | 1225 | 1270 |                   |
| $\frac{1}{12}$ | 550 | 825  | 1100 | 1375 | 1627 |                   |

Many of the higher-power objectives are provided with a movable collar, by means of which correction for the thickness of the cover-glass may be made. By means of the collar the distance between the front and the back lenses can be increased or decreased at will. In many cases lenses are sent out without an adjustment collar, but corrected for cover-glasses of stated thickness. The wider the angular aperture of an objective, the larger the amount of light admitted, and the greater the resolving power of the objective. Wide-angle lenses, however, whilst most useful for certain classes of work, are not particularly necessary for brewers' purposes; in fact, the objection that they possess very little depth of focus practically counterbalances their high resolving power.

### Apochromatic Lenses and Objectives.

These lenses are most valuable for high-power work, but for the brewer the great advantage of them is that they permit of the use of a very high-power eyepiece without



breaking down the image. The lenses are, however, very expensive.

### **Immersion Objectives.**

Of these there are two sorts, water and oil immersion. When using a dry objective the light, after passing through the glass slide, the object, and the cover-glass, has to pass through a layer of air before it reaches the objective. In doing this the rays are to some extent deflected from the perpendicular, and therefore there is some loss of light. When, however, a layer of water, or, still better, of cedar-wood oil, joins the trans-lens and the objective to the cover-glass, the rays in the first case are not so much deflected, and in the second are not at all deflected; so that with an immersion objective the larger amount of light enters the objective and passes up to the observer's eye. When an oil-immersion objective is used the thickness of the cover-glass is not of very great consequence, and therefore such objectives are often termed homogeneous.

## **2. EXAMINATION OF YEASTS.**

### **Culture Yeast.**

In the ordinary examination of yeast under the microscope, the yeast should be diluted with perfectly clear water—a good ordinary water answers well, and in most cases is sufficient, and distilled water is seldom necessary. The dilution should be such that the number of cells in a field should be from 60 to 80, and the following are the characteristics of a good yeast :—

(1) Cells should be uniform in size. The presenee of very small or very large eells is a sign of weakness.

(2) The cells should be well vacuolated, with one or two nuclei; but the vacuoles should not be excessively large, nor should the nuclei be very bright. An exception must be made, however, in this last respect, in the case of stone square yeast, in which a particularly bright vacuole is a normal characteristic. Yeast of Burton type is generally somewhat oval, whilst that of London type is generally perfectly round.\*

(3) Either a very thick or a very thin cell wall indicates weakness, whilst excessive granulation is found in either aged or dead cells.

(4) The proportion of old and dead cells should not exceed 2 per cent.

(5) The proportion of bacteria should also not exceed 2 per cent., although a proportion which in a thoroughly vigorous yeast might be harmless would, in a weak yeast, constitute a serious danger.

Whilst microscopical examination of yeast will not give any certain information as to the presence of wild yeast forms, it does give information as to the extent of bacterial infection. It can also be ascertained if the sample is clean, or whether it contains much protein or sludge matter. Hop resins may also be present, and are easily distinguished by the fact that the preparation, when treated with a drop of very dilute ammonia, will not show these resinous bodies, they being soluble in the alkali.

Starch, when present, may readily be detected by the blue colour produced when a drop of iodine solution is added to the preparation.

\* Many pitching yeasts are of composite type and contain both oval and round cells.

### Staining.

In order to facilitate the examination of certain objects staining is sometimes resorted to; but, on the whole, it is probably better not to stain under ordinary circumstances, save in the case of particular bacteria, for the staining of yeasts and moulds frequently entirely obscures their internal structure. The identification of dead and weakly yeasts can with a little practice be carried out perfectly well without the aid of stains. In the case of certain bacteria, however, staining occasionally is very useful, and for particular organisms special stains are required. Full details of these may be obtained in many of the works dealing specially with the subject.

### Vacuole and Nucleus.

Reference should be made to an important paper on "The Yeast-cell," by H. Wager,\* in which the author describes experiments which lead him to conclude that what has been known heretofore as the "vacuole" of the yeast is actually the "nucleus," and what was known as the "nucleus" the "nucleolus."

This particular vacuole or nucleus is permanent in the cell, and where others are at the same time formed, these remain as true vacuoles, increasing or decreasing in size according to the particular condition of the cell's activity. The same author has further investigated the *glycogen of yeast*, and he shows how "the appearance of a number of vacuoles at a certain stage in the development of the yeast plant is largely due, if the yeast is healthy, to the formation of glycogen," and when the glycogen disappears the vacuoles always disappear, with the exception of the one vacuole which we may call the "nuclear vacuole." He further remarks that "the glycogen is extremely variable in amount, and seems to have an important connection with the fermentative activity of the yeast. . . . for example, during the progress of fermentation, as the glycogen increases in quantity, the cells lose their fermentative activity to a large extent, and gradually sink to the bottom of the liquid."

### Chemical Analysis of Yeast.

Brewers' yeast, in a condition for pitching, contains about 85 per cent. of water, and pressed yeast 70 to 75 per cent. No obvious advantage is obtained by making a chemical analysis of yeast, but it should be mentioned that the organism is made up of cellulose as

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\* *Journ. Inst. Brew.*, 1911, pp. 2-15.

chief constituent of the cell wall, with internal protoplasm of proteins, fat, and mineral matter. The following analyses give the average composition of dry yeast and its ash :—

| <i>Dry Yeast (Nägeli).</i> |     |     |    | <i>Mineral Matter (Lintner).</i> |     |     |      |
|----------------------------|-----|-----|----|----------------------------------|-----|-----|------|
| Cellulose                  | ... | ... | 37 | Silica                           | ... | ... | 1.3  |
| Protein—                   |     |     |    | Iron ( $\text{Fe}_2\text{O}_3$ ) | ... | ... | 0.5  |
| Albumen                    | ... | ... | 36 | Lime                             | ... | ... | 5.5  |
| Gluten casein              | ... | ... | 9  | Sulphuric anhydride              | ... | ... | 0.5  |
| Peptone                    | ... | ... | 2  | Magnesia                         | ... | ... | 6.1  |
| Fat                        | ... | ... | 5  | Phosphoric anhydride             | ... | ... | 50.6 |
| Ash                        | ... | ... | 7  | Potash and trace of              | ... | ... |      |
| Extractive matter          | ... | ... | 4  | soda                             | ... | ... | 33.5 |
| <hr/>                      |     |     |    | <hr/>                            |     |     |      |
| 100                        |     |     |    | 98.0                             |     |     |      |

The protein or total nitrogen on dry yeast varies considerably. It may be as low as 40 per cent. or as high as 60 per cent.

It will be inferred from the above analysis that for a yeast to remain in healthy condition and to maintain its particular type and properties the balance of phosphate and nitrogen food must remain in something like a constant proportion, and that therefore the food supply of the yeast must be as uniform as possible.

E. R. Moritz\* has given it as his opinion that one of the most influential factors in the transmittable characters of a yeast is the gravity of the wort.

He points out that the gravity represents the relative amount of food and of chemical work to be done, and also of fermentation products formed per unit of volume, while it also effects the viscosity of the liquid and amount of heat liberated—it having therefore a chemical, physical, and biological effect.

Much important work by Adrian Brown has been completed dealing with the various factors of fermentation as they affect yeast, and the student should carefully study his various papers in the *Journal of the Chemical Society* and elsewhere on the subject.

The treatment of yeast when skimmed or otherwise removed from the beer for future pitching purposes is important. The head of yeast is often collected at somewhat high temperature, and this should be

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\* "The Transmitted Tendencies of Brewery Yeasts," *Journ. Inst. Brew.*, 1903, pp. 222-245.

brought back as quickly as possible and stored at temperatures well below 60° with access wherever possible to currents of pure air.

## Secondary and Wild Yeasts.

Several methods have been devised for detecting the presence of wild yeasts, the chief of which are :—

1. The spore formation method, depending upon the power these organisms have of forming ascospores under certain conditions of temperature and in different times.

2. A method of analysis, by means of which the yeast is sown into a prepared gelatine wort in such way as to allow of each separate yeast-cell forming a colony of growth in the gelatine wort, with subsequent microscopic examination of the pure colonies.

3. By method of film growth.

4. A method in which the sample of yeast is sown into sterilised beer.

The detection of secondary or wild yeasts in a culture sample is not possible by simple microscopic examination, and resource must be made to one or other of the above methods when this is required.

**Spore Formation Method.**—Generally speaking, wild yeasts will form spores much more rapidly than will culture yeasts; and working under definite conditions of temperature and time, some approximate idea as to the percentage of wild yeasts present in the culture sample may thus be obtained. Hansen, Jörgensen, and others have tabulated a series of times and temperatures for the spore formation of various types; and where it is desired to identify different types of these yeasts, very elaborate and careful work is required.

Hansen's work was carried out largely with low-fermentation or bottom yeasts, and the method is perhaps not so satisfactory a one for testing our top-fermentation yeasts; nor, in the writers' experience, is there always the sharp dividing line of sporulation which might be expected. And, whilst it must be admitted that satisfactory results depend largely upon the particular thickness and general conditions of the yeast as spread upon the gypsum block (see p. 378), yet it has certainly been found that under some conditions satisfactory sporulation of some wild yeasts commonly found in English beer deposits, as, for example, *Sacch. ellipsoideus*, will form spores only in a small percentage, and with considerable difficulty; whilst, again, some culture yeasts will not spore at all—or only very slightly—even after several weeks. Certain types of culture yeast do, however, in the writers' opinion, form spores fairly freely when treated by Hansen's method, and these appear to be yeasts of somewhat oval and attenuative type. But it has been found very difficult, in certain definite cases, to produce spores at all, particularly from the well-known round London yeast.

Certain forms or varieties of torula type appear to approach closely the actual yeast type—so closely as to make it a difficult matter to decide whether the organism is a torula or true yeast. As is well known, the usual distinction between these two classes of cells is that the yeasts form spores and the torulæ do not. But no doubt this distinction is largely lost in organisms which are intermediate between these two classes. In the same way, certain mould forms show very great similarities to true yeasts.

According to Jörgensen, it is important, when examining



a culture yeast for the presence of wild forms by the method of spore formation, that the sample be taken under suitable conditions and at the right time. Thus, for example, he advises that the yeast be taken neither from the surface nor from the sludge at the bottom of the fermenting vessel, as such will either not form spores at all, or will only form them to a very slight extent. He advises that the yeast to be examined should be taken when fermentation is vigorous, and whilst the cells are suspended in the beer.

As, however, it is generally more practicable to examine a sample of the yeast which has been collected in the yeast-back preparatory to pitching the subsequent gyle, it is desirable that such a yeast should first be invigorated by growing some in a little sterile hopped wort, previous to sowing on the gypsum blocks.

**Sterilised Hopped Wort.**—This is used not only for the present purpose of invigorating a culture yeast previous to sporulation, but also for the examination of such materials as sugar, beer deposits, etc., where a sterile medium is required for detecting yeast-cells, bacteria, or other organisms.

A wort of about 1050 gravity is obtained either ready hopped from the brewery or from a malt mash obtained in the laboratory and afterwards hopped at about 8 lbs. per quarter of malt. The wort is boiled in the ordinary way in a large flask for one hour, cooled to 60° and filtered. It is then reboiled, cooled back, and again filtered, and it will be found necessary to do this several times before a sufficiently bright wort is obtained.

Small flasks of about 70 c.c. capacity are carefully cleaned, rinsed with distilled water, and finally sterilised by plugging with cotton wool, and boiling a little distilled water in the plugged flask for a few minutes. Several flasks may be boiled together in this way on the sand bath, and the steam allowed to thoroughly percolate through the wool plug. The flasks are then filled about half full with the hop



wort and boiled for a few minutes. The boiling is repeated again on two alternate days, and the worts when so prepared should be fairly bright and without sediment when cold. They are then ready for use.

A platinum wire or needle, sterilised by heating in a Bunsen flame for a minute or two, is allowed to cool for a moment, and a small sample of the culture yeast to be examined for wild types transferred by means of it to one of the flasks containing the sterilised hop wort. The yeast is quickly mixed into the wort, and the cotton-wool plug at once replaced. The wort is then kept for 24 hours at the ordinary temperature, about 60° F., poured off, and a fresh supply from another flask poured on to the yeast, and the flask now kept at a temperature of about 80° in the forcing cupboard for a further 24 hours.

Whilst the yeast is being grown and invigorated in this way, gypsum blocks are prepared for sowing the yeast, so that it can grow under sterile conditions without nutriment. The gypsum or plaster of Paris blocks are generally of cone shape, with a flat top, and these are first prepared by carefully scraping clean the flat surface, and afterwards sterilising in a covered glass dish at a temperature of 230° F. for an hour.

When the yeast is ready for sowing (after 48 hours), the gypsum block in the dish is allowed to cool, and, as a preliminary precaution, a little alcohol may be lit on the surface of the gypsum just before spreading the yeast, to make quite certain of complete sterilisation.

The wort is now poured off from the yeast, the glass cover containing the block is partially raised, and a little of

the liquid yeast poured on to the block, and quickly spread over the surface by means of a sterilised glass rod. Some recently boiled, cold distilled water is poured into the dish until this is about half filled, the cover is quickly replaced, and the culture transferred to the forcing cupboard at a temperature of 80° for 48 hours, after which time a little yeast is withdrawn from the block, diluted with a drop of distilled water, and carefully examined under the microscope.

Under these conditions, wild yeasts will spore fairly readily, whilst healthy yeasts will not spore until much later.

Hansen further points out that the spores of wild yeasts may be distinguished from those of culture yeasts by the fact that the former have an indistinct cell wall, with clear, highly refractive, and uniform contents. Culture yeast spores, on the other hand, are larger, have a distinct cell wall, and usually show their contents somewhat granulated, and often vacuolated.

The method of spore formation is generally a useful one for obtaining some information as to the character of the pitching yeast.\*

**Method of Analysis by Sowing into Gelatine Wort.**—This method has some disadvantages, one of which is that it is difficult to make sure, unless a very dilute solution of the yeast is taken, that each colony afterwards growing on the gelatine wort is the result of a single yeast-cell. But the method is, on the whole, a

\* See also "On Spore Formation among the Saccharomycetes," by B. T. P. Barker, *Journ. Inst. Brew.*, 1902, pp. 26-72.

very useful one for analysing out a sample of yeast. The wort gelatine is prepared as follows :—

**Preparation of Wort Gelatine.**—To prepare a 10 per cent. gelatine wort, take 100 grammes of the best gelatine (Coignet sheet gelatine is the most satisfactory), and add this to 1 litre of hopped wort of about 1050° gravity, the preparation of which has already been described on p. 377. Dissolve the gelatine in this by heat and continual stirring, best carried out in a large porcelain dish placed for two hours over a boiling water bath.

The gelatine should be torn up into very small pieces before dissolving, and the wort gelatine is afterwards filtered through an ordinary filter paper fitted into a water jacketed funnel, which must be kept sufficiently heated to allow the gelatine preparation to filter through readily into a large, scrupulously clean flask, and, whilst so filtering, a plug of cotton wool should be arranged round the stem. It is returned to the filter until it comes through quite bright; and when as much as possible has filtered, the preparation is made up to 1 litre (equal to 10 per cent. gelatine solution). The flask containing this is then plugged tightly with cotton wool, and sterilised in a steam bath for 20 minutes, allowed to cool, and again sterilised on two alternate days in the same manner.

When it is required to fill test-tubes with the wort gelatine, this latter must be carefully melted by standing the flask in warm water (too high a temperature being avoided, as, after a time, this would tend to prevent the gelatine resolidifying). Carefully sterilised test-tubes are filled to about one-third of their length by withdrawing the required amount of wort gelatine from the flask by means of a pipette, and running this into the test-tube in such a way as to prevent the gelatine touching the sides or top of the tubes; they are at once plugged tightly with cotton wool, fitted with indiarubber caps, and sterilised in a steam bath on two alternate days.

The yeast is suitably diluted, and the drop transferred to the just-melted wort gelatine contained in the test-tube. This is thoroughly shaken into the gelatine, and rapidly transferred to a sterilised Petri dish, on which the cover is at once placed, and the dish so manipulated as to obtain the gelatine wort in a uniform layer over its surface. It is then

set aside in a cool place until the colonies of yeast are sufficiently developed for examination. Each colony can be separately examined under the microscope, and, if necessary, re-sown in sterile wort for a more vigorous reproduction.

Another method is that of spreading the diluted yeast solution, by means of a paint brush, across the surface of the solid gelatine wort in the Petri dish. Two or three of such spreadings may be made, using different dilutions of the yeast, and in this way the precaution is taken that the yeast is sufficiently dilute to allow of the colonies growing separately in the wort gelatine.

Care must be taken, in examining colonies under the microscope, that samples of each colony are taken with the platinum wire from several different parts, since the form of the cells as taken from the edge of the colony where this is in contact with the gelatine will often show considerable differences from the cells as taken from the centre.

**Film Formation.**—This method depends upon the fact that, when certain yeasts or bacteria are grown in nutrient liquids, such as malt wort or beer, such liquids remaining in a perfectly quiescent state, but being at the same time exposed to germ-free air, the organisms will tend to form what is known as a film growth upon the surface of the liquid, and such film examined microscopically will be indicative of the type producing them.

The method is not very suitable for a general working laboratory, and considerable experience is essential for the

method to be of much value in the detection of secondary yeasts—it is, perhaps, of more value for confirming the character of a pure culture.\*

**Sterilised Beer Method.**—This depends upon the fact that many secondary types of yeast will only grow freely when the activity of the primary or culture yeasts has sensibly declined. The method is simply and easily carried out, and gives useful indications as to the general condition of the yeast, both bacterially and also from the point of view of wild yeast infection. Sterilised beer is required, and is prepared as follows :—

An ordinary running beer taken from a brewery is fined or filtered bright, and filled into ordinary Pasteur flasks, previously sterilised at  $250^{\circ}$  for an hour, these being then corked, and the beer in them just brought to the boil by heating on a sand bath. The bent tube should be plugged with a small piece of cotton wool, afterwards allowed to cool, and sterilised in a similar way on two alternate days. In place of the Pasteur flask, the beer may be filled into small sterilised bottles, the cork tied down, and, after filling, the beer sterilised in a steam bath for 20 minutes on two alternate days.

A little of the pitching yeast to be examined is transferred by means of a sterilised glass rod to the sterilised beer, thoroughly mixing it in, and the latter forced at  $80^{\circ}$  for two weeks. Under these conditions, such secondary

\* A. C. Chapman and F. G. S. Baker have published a series of excellent photomicrographic illustrations of both the common and rarer yeast species in film formation, ascospore and sedimentary types, in the *Brewing Trade Review*, 1905 and 1906.

yeasts as may be present will tend to grow and ferment the beer, and at the end of this time the deposit is examined, and in this way some approximate idea as to the amount of wild yeast present in the original pitching sample may be ascertained.

As was the case with the spore formation test, it is advisable, before adding the yeast to the sterilised beer, to first invigorate this for 24 hours in sterile wort.

Hansen's and Jörgensen's work upon yeasts has already been referred to, but the following may also be mentioned :—

The Canton Lectures on Yeast. By A. Gordon Salamon.

*The Student's Manual of Yeast Culture.* By Grove Johnson.

"The Influence of Light and Spectral Colours on the Sporulation and Vitality of Yeast." By Purvis and Warwick. *Journ. Inst. Brew.*, 1908, p. 214.

### 3. EXAMINATION OF BEERS.

#### Forcing Tray Test.

This test was devised by Horace Brown, and consists of maintaining the beer at a temperature of about 85° F. for a given period, so as to accelerate any changes which are likely to occur during prolonged storage at ordinary temperatures. The test is of great practical value, and is very largely employed in brewery laboratories, for by it we are able to gain "advance" information of the stability, brightening, and conditioning power of beers, and are therefore able to discriminate as to which beer of a number of brewings it is best to keep and which it is wiser to send out without delay.

In order to carry out the test, samples of beer should be taken, either from the racking vessel at the time the casks



are filled or from the casks themselves, or, still better, from both. It not infrequently happens that samples from racking tank exhibit a greater stability than those from cask, and this may be easily explained by the fact that casks cleaned, and even steamed, in the ordinary way are rarely actually sterile. On the other hand, it will sometimes happen that the stability of beer drawn from cask is greater than that from racking vessel. This, when found to be the case, is usually due to the presence of hopping-down hops, the powerful preservative influence of which is thus demonstrated.\*

### The Forcing Tray.

The "forcing tray" is itself well known. It is usually a copper vessel some 3 or 4 inches deep, with a flat top, and of any convenient size. A measurement of 2 feet 6 inches long and 1 foot broad is suitable. This vessel is nearly filled with water, and the whole is placed in a cupboard and supported by a stand or bracket. An ordinary gas burner (not a Bunsen) is placed a convenient distance below the tray, and is connected with a gas regulator, by means of which a constant temperature may be maintained on the tray. A Page's, or, still better, a Reichert's, regulator may be used. In these regulators the gas has to pass through an opening in a glass tube, the length of the opening being regulated by a column of mercury, which, expanding as the temperature rises, reduces the aperture and cuts off a portion of the gas passing through the regulator

\* The value of hopping-down hops in this direction is proportionately greater than when added to the copper. Thus, for example, if 8 lbs. of hops are used per quarter, we get a more stable beer by employing 6 lbs. in the copper, and  $\frac{1}{2}$  lb. per barrel in cask, than by adding 7 lbs. in copper and  $\frac{1}{4}$  lb. per barrel in cask. This would seem to point to the desirability of a reduction of copper and an increase of cask hops; but there are obviously limits to the amount which can be added to cask, and the value of such hops is only experienced when beers are stored and rolled sufficiently to permit of their extraction.



to the burner; whilst, on the other hand, if the temperature falls, the level of the mercury is lowered, and a larger current of gas is permitted to pass to the burner. A pinhole perforation in the side of the tube secures the passage of a small quantity of gas, so that in case of a sudden rise in temperature, and consequent increase in the height of the mercury, the gas flame shall not be completely extinguished. The height of the mercury is regulated by means of a screw which raises or lowers the column.

A constant temperature of 85° F. should be maintained. It will require some adjustment of the regulator to secure this, but having ascertained the correct adjustment, the regulator should not again be touched. The bulb of the regulator (filled with mercury) is, of course, inside the cupboard, but does not actually dip into the water of the tray. To ascertain the temperature of the tray, it is best to keep a small thermometer immersed in a flask of water standing on the tray. This is better than inserting the thermometer in the water contained in the tray itself.

The beer is placed on the tray in special flasks. These hold about 80 c.c., and are provided with a side tube, projecting from and at right angles to the neck. This tube is bent downwards at about 2 inches from the neck, the end dipping into a shallow dish of mercury, which acts at once as a valve to allow escape of gas, and prevents entrance of air. A small glass evaporating dish answers well as a receptacle for the mercury, and a number of flasks may be placed round it, the tube of each dipping into the mercury. The flasks must be carefully sterilised before they are used. To do this, pour in a little strong sulphuric acid, turning the flask round so that the acid touches each portion of the interior, including the side tube. Pour this acid from one flask to another, and in this way many flasks may be cleansed with the same acid. After rinsing the flasks thoroughly, place a little distilled water in each, insert an indiarubber stopper in the neck, and boil the water in the

flask until steam has blown through the side tube for about a minute. Remove the flask from the flame, pour out the water, and, immediately it is cool, fill with the beer to within half an inch of the level of side tube in the neck of the flask.

Another means of preparing the flasks, which is in many ways preferable, is, after cleaning them carefully with acid and water, to place them (without the corks) in the hot-air oven or steriliser, and to heat them to 200°—250° F. for about twenty minutes. A good quality ordinary cork is then passed through a flame and inserted in the flask immediately it is taken from the hot-air oven. After allowing to cool, the flasks are filled in the usual way.

The length of time during which the beer should remain on the forcing tray will depend upon the particular class of beer that is being tested; thus the quick-running mild ales or porters of London and large towns should not require more than three or four days' forcing for giving sufficient indication as to the stability of the beer.

Bitter beers and mild ales generally, however, should be kept for seven days on the tray; whilst stock ales and export beers should have three or even four weeks' forcing.

Before the beer is placed on the forcing tray its specific gravity should be taken, and a careful examination of it should be made. If this is a racking sample, it should be noted whether the beer is fairly clean or if it shows a tendency to drop fairly bright readily; and it may be also advisable to test its fining capacity. If the beer is in the fined condition, then it should be noted whether it is per-

fectly brilliant or shows a slight opalescent haze. In connection with haze, it should be remembered that a fined beer from cask, when filled into bottle for sampling purposes, may often become piecey from having been bottled too soon after fining ; and, further, a slight haze may be due to the same cause, or to slight drop in temperature.

On taking the beer off the forcing tray, the following observations should be made :—

1. As to its condition of brightness and flavour.
2. As to the amount of deposit and its microscopical examination.

The specific gravity, and, if necessary, the acidity also, should be determined.

### Condition of Brightness and Flavour.

If the beer remains brilliant after forcing it is a satisfactory indication, as if infection from bacteria or wild yeast is considerable there would almost certainly be a haze produced ; although, on the other hand, the beer may have become quite sour, and still remain bright.

If the beer is hazy, a small quantity should be tested with a drop of ammonia, or shaken up with ether, when, if the beer becomes bright, it will indicate that the haze was due to resinous matter. A nitrogen haze usually disappears on warming the beer.

The flavour should be carefully noted, though it is not so easy to judge as to this on a forced sample ; but any rank, bitter flavour, due to unsatisfactory hops, or the persistent bitter back flavour of permanent character due to yeast bite, which is easily distinguished from a hop

bitter, should be noted ; and, if there is marked acidity in the flavour, this should be estimated by means of N/10 alkali.

If there is a heavy deposit from an unfined racking sample, this would suggest either that the beer was insufficiently clean when racked, or that there has been a considerable growth of yeast produced, which latter would be confirmed by a difference of several degrees of gravity before and after forcing.

This latter may be due to fermentation not having proceeded normally in the brewery, and to the beer not attenuating well, or it may possibly be due to the addition of a heavy quantity of priming, whilst, to some extent also, it depends upon the type of yeast, the particular mashing heat used, and other causes.

A healthy beer will generally lose 1-2° gravity when forced for about seven days, but some beers will remain unaltered in gravity for a fortnight or even three weeks, and this is more often the case with stock ales or heavy gravity beers or stouts, than with beers of 17-18 lb. gravity.

When a beer does not attenuate on the forcing tray it is obviously in a condition of greater liability to bacterial growth, since it is without the protective activity of the primary yeast, which may be due to lack of nourishment, in which case the culture yeast will appear dead and granulated.

At the same time, it must be carefully borne in mind that the condition of the beer when in the forcing flask is very different from that in cask. In the forcing flask, not only is beer kept at a higher temperature, but it is

under little or no pressure, and is without, therefore, the naturally protective carbonic acid gas dissolved in the beer. At the same time, the beer in cask, being under conditions of larger bulk, and in wood, will more readily allow a secondary activity of the primary yeast to commence than when the beer is in flask. These points are the more important if the beer tends to be flat; as, under these conditions, the quiescent state of the beer is more dangerous in flask than it would be in cask, for the reasons which have been pointed out.

The forcing method, as an indication of beer stability and quality, is of the utmost value to the practical brewer, but great care should be taken in interpreting results obtained by this method that, as far as possible, the life history of the beer, so far as this is known, be also taken into consideration.

### Examination of Deposit.

**Secondary Yeasts.**—If the total amount of these does not much exceed 5 per cent. no serious danger is to be feared in a beer which is for moderately quick consumption, particularly if such beer remains perfectly bright and was originally a fined sample. The presence of wild yeasts in an unfined racking sample is more serious; if the amount is excessive the beer will often refuse to fine quickly—a most important point with certain beers—or it may fine at racking, but afterwards develop a persistent haze, due to the wild yeast cells remaining suspended in the beer, during which time it is quite impossible to fine the beer bright.

The primary yeast cells will fine out readily from the beer, but the wild forms will, in this way, remain suspended; and the early hazing of bottled beer is often due to this cause.

Most wild-yeast infection is aërial, and takes place during the cooling of the wort on the refrigerators, or, if the temperature will allow, on the coolers. Where infection has resulted from dirty mains or brewing vessels, the beer deposits will usually show not only several different types of wild yeasts, but also bacteria and amorphous matter. The infection on the refrigerator will more likely show the presence of one or more distinctive wild yeasts.

Infection of wild yeasts will also frequently occur from the infected wood of fermenting vessels, etc.

The following short notes refer to a few of the chief secondary yeasts, bacteria, etc., found in English beer deposits:—

*Saccharomyces pastorianus*.—There are three *Pastoriani*, though only one commonly found in English breweries. This is a frequent source of infection, producing cloud and fret. If the beer is otherwise sound, however, it may after a time recover and become bright and drinkable, though probably thin in palate.

The familiar elongated appearance of these yeasts is apt to change. When active, the cells tend to become smaller, shorter, and more like mycoderma cells; and the yeasts of this class are characteristically variable in size—they can, of course, ferment malto-dextrins.

*Saccharomyces ellipsoideus*.—This is a very common form of wild yeast, and frequently found in infected beer



deposits. It is much smaller in size than culture yeasts, and easily distinguished from them. The yeast frequently gives rise to a sick fret, sometimes accompanied also by stench; and when a beer is in a "fretty" condition, due to this yeast, it is impossible to fine it bright until the fret is over. The beer may remain sound and palatable, but in the case of a beer for quick trade is generally then too old and thin in flavour to be saleable.

*Caseous Yeasts*.—These yeasts are sometimes met with in beer deposits. They are distinguished by the peculiarity they possess of conglomerating together into cheesy masses, which fall to the bottom of the beer. It is not supposed that these yeasts are of any particular danger to the beer.

*Saccharomyces apiculatus*.—This yeast is rarely found in beer deposits, and arises, as is well known, from the skins of fruits, and gives rise, when present in sufficient quantity, to a vinous fermentation. It has the familiar and well-known appearance of oval cells with pointed ends.

*Mycoderma*.\*—These organisms are frequently met with in beer. They are liable to grow both as an aërial organism on the surface of the beer in contact with air or submerged. When in this latter condition, they may set up some amount of fermentation, and the cells are somewhat larger and more clearly vacuolated than when functioned as an aërobial ferment. There are a number of mycoderma forms, such as *Mycoderma cerevisiæ*, *vini*, and others.

\* "*Mycoderma cerevisiæ*," by H. Van Laer, *Journ. Inst. Brew.*, 1901, pp. 337-356.



*Moulds*.—These may obtain access to the beer from materials such as dry hops, or from dirty vessels, and also, of course, from the air. Mould spores can produce little, if any, fermentation, and unless they give rise to a large amount of growth on the surface of a flat beer are of no danger.

*Brettanomyces*.—This organism was discovered by Claussen as a secondary yeast in stock beers, and he states that he has proved experimentally that this secondary yeast definitely exists, though it is not a *saccharomyces*, but a non-sporulating budding fungus or *torula*. He considers its action absolutely necessary for bringing English stock beers into proper cask and bottle condition, and for imparting to them the peculiar flavour characteristic of such beer. He states that it is present to a small extent in most English pitching yeasts, that it works very slowly and settles to the bottom, and that its distinctive character from other *torulæ* is in the fact of its being able to form a much larger quantity of acid. As in the case of ordinary *torula* yeasts, the fermentation produced is very slow.\*

*Torulæ*.—These are also occasionally found in yeasts and beer deposits, but little is known of their action. Some *torulæ* undoubtedly produce turbidity of beer. Others appear to have no distinctive action.

*Bacteria*.—The common, acid-forming bacteria usually found in a beer deposit are the lactic, acetic, and, more rarely, butyric. Whilst it is not possible to distinguish

\* *Journ. Inst. Brew.*, 1904, pp. 308-331; also "On a *Torula* in English Beer Manufacture," by H. Schiönning, *Journ. Inst. Brew.*, 1909, pp. 2-35.

each of these acid forms of bacteria by a purely microscopical examination in a yeast, it is more easy to do this in a beer deposit, and, further, it should be noted that if the acidity in the beer, due to the growth of any of the above organisms, is sufficiently advanced, the differences in palate flavour are quite noticeable—thus, with lactic acid, there is an absence of the sharpness found with acetic. Butyric acid is easily distinguished by its characteristic smell, but is rarely found in beer in sufficient quantity to allow of this. The following is a brief description of the various bacteria commonly found in beer deposits:—

*Bacterium lactis* (lactic acid ferments).—This acidity is usually associated with two different kinds of lactic bacteria, both of which are constantly met with in beer deposits. The one known as the lactic acid bacterium, or Pasteur's lactic ferment, is distinguished by its 8-shaped figure, it being extremely small, and sometimes occurring in chains. The other is known as *Bacterium lactis*, and this occurs in the form of short rods of about 2 to 3  $\mu^*$  in length, and the maximum activity of this ferment takes place at about 120° F. But, at any temperature above 70° F., this bacterium will become extremely active. This is an important point in connection with fermentation heats, as, if the maximum temperature is above 70°, there would be considerably more lactic acid production than would be the case supposing the maximum fermentation temperature not to rise above 66° to 68°.

*Bacterium termo*.—A small bacterium, having a figure-of-

\*  $\mu$  is the symbol used for a micro-millimetre =  $\frac{1}{1000}$  mm.

eight appearance, due to a constriction in the centre of a short rod, measuring about  $1\mu$ . It is practically the ferment of putrefaction, and, according to Cohn, is always present wherever putrefaction is occurring. Seldom found either in beer or yeast, but frequently in zooglea formed in slime on cellar walls, etc.

*Bacterium butyricum*.—Also sometimes termed *Clostridium butyricum*. Rods slightly oval, having a length of about  $4\mu$ , a width of  $1\mu$ . Not infrequently found in beer deposits, especially in those of beer brewed in old and dirty plants.

The organism is also found in putrid grains and decomposing spent hops, to which it gives its peculiar and very characteristic odour.

*Bacterium aceti*.\*—The organism appears characteristically in deposits in the form of chains, from 1 to  $2\mu$  in length, each separate organism being of diplococcus form and slightly constricted in the middle, frequently giving them a figure-of-eight appearance, though not so pronounced as in *Bacterium termo*. There are at least three species of bacteria which produce acetic acid. Most favourable temperature is from  $90^{\circ}$  to  $95^{\circ}$ . At temperatures below  $60^{\circ}$  its action is very slight. An ample supply of free oxygen is necessary for its growth, hence acetic acid is frequently found in beer in half-empty barrels or bottles, but seldom in that contained in full vessels.

*Saccharobacillus pastorianus*.—Exists as long rods, sometimes straight, sometimes curved, occasionally jointed. Width

\* For a full investigation of this bacterium by Adrian Brown, see *Journ. Chem. Soc. Trans.*, 1886, p. 172 ; and 1887, p. 638.

about  $1\ \mu$ , length from 4 to  $10\ \mu$ , sometimes much longer. Very frequently found in unsound beer deposits, generally associated with dirty plant. Stated by some observers to produce lactic acid. It is curious, however, that some beer deposits will be practically swarming with this ferment, and yet the beer may be free from any abnormal acidity.

Its most characteristic property is that of producing a very definite thinness of palate in a beer, which may or may not be associated with acidity. It is sometimes found in samples of pitching yeast, and often finds its home in casks in which the wood has become considerably softened. The organism is very resistive of high temperature, but is not to be confused with *Bacillus subtilis*, obtained from hay infusion, and which is itself quite a different organism.

*Sarcina*.—Lindner has described six species of sarcina, but that found in English breweries is probably the *Pediococcus cerevisiæ*.

Actual sarcina organisms should be cube-shaped, but such are rarely found in an English beer deposit, and they more often occur in groups or packets of four cells as tetracocci. When in groups of two they are known as diplococci. When these latter are present it is very unusual to find the beer actually ropy, and often there may be a great number of diplococci forms in a deposit without any tetracocci or other sarcina types, but when the diplococci forms are present, the beer has a tendency to ropiness and to the formation of tetracocci, a development which is more pronounced after storage in bottle than in cask.

Sarcina action is not clearly understood, but its presence is often accompanied by the production of cloudy beers

possessing a distinctive and very characteristic odour, and, of course, it is frequently found, but not always, in ropy beers, though it has not been proved whether it actually is the direct cause of ropiness.

These organisms unquestionably tend to develop most in beers of abnormally low acidity. This may be due to a low percentage of acid phosphates or may be due to want of actual lactic acidity, but there is almost certainly a connection between the presence of phosphates and sarcina.

Sarcina is often introduced by malt, hay, and chaff dust, occasionally also rotten wooden vessels, and when once these organisms have obtained a hold in a brewery, it is a matter of great difficulty and long time before they can be thoroughly eradicated. The use of raw sugar as priming or in the copper certainly predisposes a beer to sarcina infection, and other predisposing causes are under-cured and slack malts.

All varieties of sarcina are said to be destroyed at a temperature of 140° F., but it is safer not to attempt to blend back a definitely sarcina-infected or ropy beer into a gyle even after this has been sterilised, for the reasons given above.

## BACTERIOLOGICAL EXAMINATION OF WATER.

The two tests described are those of Hansen and Koch. In Hansen's test a water is held to be a satisfactory one if, when subjected to this test, the wort to which it has been added remains clear after three days' exposure to a temperature of 90° F. By Koch's test a water is held to be

pure which does not yield more than 100 colonies of organisms per centimetre. It must be remembered that the type of organisms present is often of far more importance than the actual number, and that, although bacteriological tests are valuable as confirmatory evidence, they must not be relied upon too exclusively.\* Some very pure deep-well supplies contain many organisms. Again, much depends upon the exposure to the air to which the water has been subjected. Thus water which has been standing in a tank or reservoir always contains a considerable number of organisms. The efficiency of filtering media is well tested by means of this bacteriological method, but the filtration of water as usually carried out is, as a rule, of little value; often it is actually dangerous, for whilst at first such filters may, and in many cases do, remove the whole or the greater part of the organisms, they require frequent renewal, otherwise the organisms retained in their pores pass through into the filtrate, and the filtered water may become more impure than the original sample. Probably the best filtering medium for commercial purposes is coke, and Salamon and Mathew† have shown the importance of the presence of iron in coke, without which its purifying power is much reduced.

\* It must not be assumed that the colonies of organisms thus found actually represent the whole of those present in the water. By no means is this the case. The test only shows the number of organisms capable of growing in the particular medium used, and under the precise conditions of cultivation to which the sample has been subjected.

† *Journ. Soc. Chem. Ind.*, 1886, p. 261.



## Koch's Method.

Sterilised wort or meat gelatine\* is inoculated with a given quantity of water, with which it is thoroughly mixed. The liquid gelatine is then poured on to sterile Petri dishes, as described for yeast analysis on p. 380, when in a few days the organisms present in the water grow, forming "colonies" on the gelatine. The number of these colonies is then counted, and the colonies of organisms per cubic centimetre of water noted. This method, though yielding concordant results, is open to the very serious objection that many of the organisms which so grow would not grow in ordinary wort or beer from which oxygen was removed, whilst, on the other hand, many organisms which would grow in wort or beer refuse to grow in the nutrient gelatine. For this reason, and because the number of organisms present in impure water is so fluctuating, the method of Koch should merely be employed when a very complete examination is being made under special conditions, and much caution must be observed in condemning a water or in passing one as pure on the basis of this test.

\* For preparation of wort gelatine, see p. 380.

Meat gelatine is prepared from the following :—

500 c.c. of distilled water.

15 grammes of solid glucose.

5       ,,       Liebig's extract of beef.

7       ,,       peptone.

The mixture is boiled for 15 minutes, then cooled and made up to 500 c.c. This is then placed in a basin over boiling water for two hours with 50 grammes of gelatine added. It is finally made up again to 500 c.c. and filtered bright, the process being completed as described for the preparation of wort gelatine.



## Hansen's Test.

This test gives results of practical value to the brewer, and is that which is recommended.

To carry this out, it is necessary to prepare tubes or flasks of sterile wort and sterile beer as already described, and full description of which may be found in Hansen's works, and to inoculate these with the water to be examined. By this system it is possible to get some idea of the number of organisms present in the water, although for the student's purposes it will be sufficient to inoculate samples of sterile wort and beer (8 or 10 c.c. being used) with 1 c.c. of the water to be examined. Place the flasks on the forcing tray, and observe the length of time occupied for the appearance of cloud or fermentation of the liquid. It is, of course, essential in carrying out any bacteriological examination that the sample of water shall have been drawn with special precautions, and into sterile vessels, and that great care be taken to sterilise any apparatus used.\*

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\* See a recent paper by Percy F. Frankland upon "The Bacteriology of Water: Its Present Position," *Journ. Soc. Chem. Ind.*, 1911, pp. 319—334.

## CHAPTER XIV.

## THE POLARIMETER.\*

WHEN a ray of light passes through certain crystals it becomes divided into two rays, and is said to be polarised. These two rays are termed respectively the "ordinary" and the "extraordinary" ray. It is possible, by a special arrangement of prisms, to completely deflect the ordinary ray, causing it to pass out of the side of the prism, and to be absorbed in its case, allowing the remaining or "extraordinary" ray alone to emerge from the crystal. In a polarising apparatus, a Nicol prism is used for this purpose. This consists of a rhombohedron of Iceland spar, bisected in the plane which passes through the obtuse angle, the two halves being then cemented together with Canada balsam. The refractive index of Canada balsam is 1.549, and less than the ordinary index of Iceland spar, which is 1.654, but greater than its extraordinary index, 1.483. When, therefore, a luminous ray enters the prism, the ordinary ray is totally reflected at the surface of the Canada balsam, and passes out of the crystal, whilst the extraordinary ray emerges alone, there being a loss of one-half of the light. Various substances have the property of rotating polarised light, and the polarimeter is an instrument for the measurement of such rotation. It consists essentially of two parts, one for polarising light, the other for ascertaining the amount of rotation which has been

\* *Trans. Soc. Chem. Ind.*, 1888, pp. 259-276.

produced by the passage of such polarised ray through the liquid to be examined. This is effected by means of a polariser and an analyser, each consisting of a Nicol prism. The former is stationary, and serves to polarise the light and remove the ordinary ray; the latter is arranged so that it can be revolved, and by such means may compensate for the rotation on its axis of the extraordinary ray of light which has occurred in its passage through the liquid to be examined. The movement of the analyser is recorded on a dial, which bears round its face a scale divided into  $360^\circ$ . Some substances rotate a polarised ray of light to the left, and are termed *lævo-rotatory*; others to the right, and are termed *dextro-rotatory*, and the minus or plus signs ( $-$  or  $+$ ) are prefixed to the readings obtained in order to indicate the direction of the rotation.

The specific rotatory power of an optically active substance in solution may be defined as the angle through which a plane-polarised ray of light of definite refrangibility is rotated by a column of liquid, 1 decimetre (100 mm.) in length, containing 1 gramme of the substance in 1 c.c.

Now it is evident that in practice a 100 per cent. solution thus indicated could not be used. We therefore employ a 10, 20, or other per cent. solution, and calculate to 100 per cent. Observations of specific rotatory power are made either with a polarimeter such as that of Laurent, for which a sodium flame is used as the means of light, or that of Ventzke-Scheibler, with which a white light illumination from oil or gas is employed. Specific rotatory power, as determined in reference to the D-ray of the solar spectrum (that is with the sodium flame), is indicated by  $[\alpha]_D$ ,

whilst, when determined by a Ventzke-Scheibler, it is indicated by  $[\alpha]_j$ . The Ventzke-Scheibler polarimeter is graduated to a scale of 100 divisions, equalling the rotation caused by passing through a solution of pure cane-sugar 2 decimetres (200 mm.) in length, containing 26.048 grammes of pure cane-sugar per 100 c.c. at  $17.5^\circ \text{C.}$  ( $63.5^\circ \text{F.}$ ). A solution of cane-sugar of the strength named has a specific gravity of 1.100, so that the readings for cane-sugar on this instrument correspond to the specific gravity of the sugar less 1.000.

For purposes of accuracy the sodium-flame instrument is preferable, though the transition-tint instruments, by working with more light, permit of the reading of darker-coloured solutions.

*Relation of  $[\alpha]_D$  to  $[\alpha]_j$ .*

To convert degrees  $[\alpha]_D$  into  $[\alpha]_j$ , multiply by 1.111, or simply add one-ninth.

To convert degrees  $[\alpha]_j$  into  $[\alpha]_D$ , multiply by 0.9, or simply deduct one-tenth.\*

The Laurent polarimeter has two scales on the body of the instrument, and also on the movable segment or vernier. The lower scale on the body of the instrument, and the lower one on the left hand of the vernier, are used only in cane-sugar estimation, and are not necessary in brewing analysis.

When the 0 of the right-hand vernier is immediately

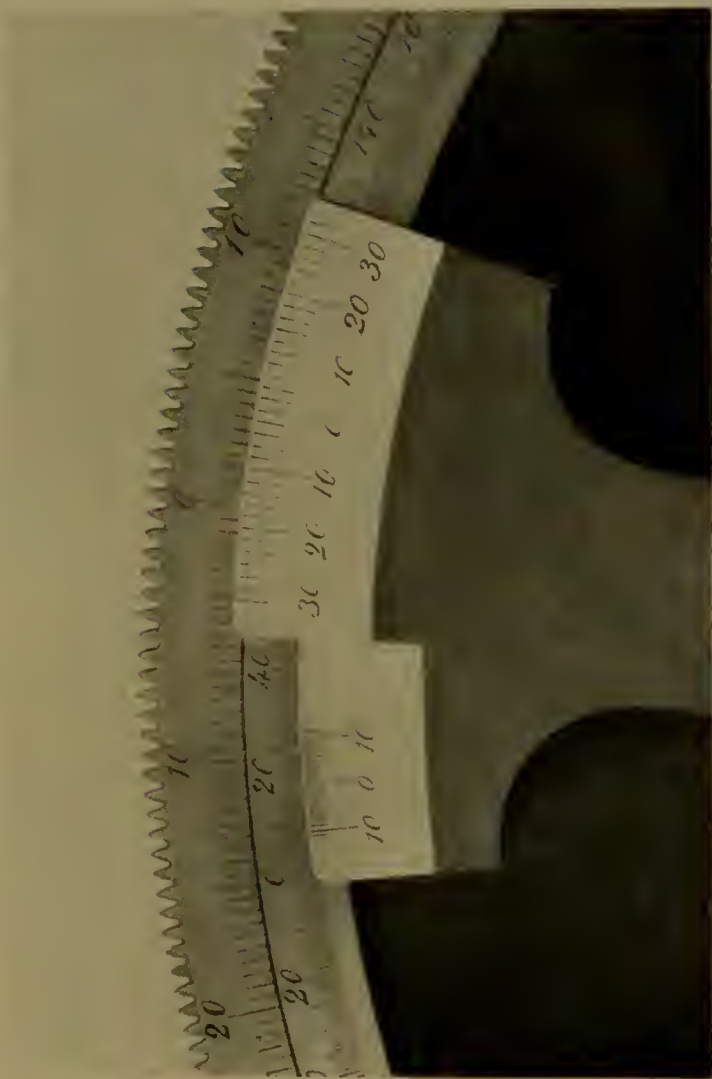
\* Brown, Morris, and Millar (*Journ. Chem. Soc.*, January, 1897) have shown that there are actually two scales of transition-tint instruments in use, which they describe as  $[\alpha]_j$  Biot, and  $[\alpha]_j$  Montgolfier. These differ slightly, but the above factors are those given by the authors for the true Biot's *jaune moyen* ray.

under the 0 of the upper scale, which is divided into degrees and half-degrees (or 30'), it is set at zero ; but this will probably need correction, as explained later on. Each division on the vernier equals 2'.

Observations should be taken with the instrument in a dark room or special cupboard, provided for the purpose. First light the lamp, placing upon the small platinum support a pellet of fused chloride of sodium. The flame will then be of a bright yellow colour. Now, looking through the instrument, bring the central line into sharp focus, by adjusting the eyepiece. Place the instrument at zero, fill the 200 mm. tube with water, taking care that no bubbles are included. Place it in the tray, and then look through the eyepiece. If the instrument is at zero, a circle of light should be seen, divided into halves by a black vertical line, and each half should be of precisely the same brightness. If not, the instrument must be corrected. This is done either by rotating the vernier until both are of precisely the same brightness, when the correction necessary is read off and deducted from or added to subsequent readings, or a permanent correction is made by means of the inner screw attached to the instrument, which rotates the Nicol prism of the analyser, without, at the same time, moving the vernier. To take a reading, the observation tube, generally made of glass (sometimes provided with a jacket to permit of any desired temperature being maintained), is filled with the liquid to be examined, taking care that the temperature is precisely 60° F. In filling, the cap attached to one end is taken off, the circular glass plate removed, and the liquid, which must be brilliant, poured in until the tube is

absolutely full—in fact, until the level of the liquid actually stands above the end of the tube. The circular glass plate is then carefully slid over the end, and the cap secured. If this is properly done, we shall then have a tube perfectly full of bright liquid, and free from enclosed air bubbles. If the liquid is dark in colour, the 100 mm. tube must be used. If sufficiently pale, the 200 mm. tube should be employed by preference, for the longer the tube the larger the reading, and the smaller the error. Replace the tube in the trough, and then again look through the instrument. Probably the vertical line will now be slightly out of focus; re-focus it by adjusting the eyepiece. The two halves of the field are now, if the liquid possesses optical activity, unequally illuminated, being darker on one side than on the other. The outer screw should now be adjusted in the direction of the darker half of the circle, until both halves of the field are evenly illuminated. It will be found at first somewhat difficult to determine with accuracy when this is the case, and it is advisable, after one or two minutes' observation, to rest the eye, and then again examine the sample. After the first experiment has been made, take the reading, then move the screw to the right or left, so as to throw the halves of the field out of equal illumination, and again adjust them. This should be repeated until two or three readings are practically concordant, and, after a little practice, this is easily attained. The polarimeter is provided with a special vernier scale, by means of which a very exact reading may be made. The plate shows such a scale.

In taking a reading, first note the number of degrees



VERNIER SCALE OF LAURENT POLARIMETER.





on the top scale indicated by the zero on the vernier scale. In the figure it will be seen that the zero lies somewhere between  $3\frac{1}{2}^{\circ}$  and  $4^{\circ}$  to the right. In order to ascertain this exact fraction, pass the eye along the vernier scale to the right until a line on that scale exactly coincides with one on the upper or stationary scale. In this instance, this occurs at 20' on the vernier scale. We have now, therefore, a reading of  $3\frac{1}{2}^{\circ}$ , or  $3^{\circ} 30'$ , as shown by the top scale alone, and, in addition to this, the vernier indicates 20', which added to the  $3^{\circ} 30'$ , gives a total reading of  $3^{\circ} 50'$ , or  $3\frac{5}{6}^{\circ}$ , or  $3.83^{\circ}$ .

In connection with polarimetry, the phenomenon of "bi-rotation" must be mentioned. By this is meant the varying optical activity of certain solutions. Thus a solution of dextrose or maltose, when freshly prepared, shows an abnormal rotation. In the case of dextrose the optical activity is above, in the case of maltose it is below, normal; but in either case it is immediately rendered normal by either the addition of a very small amount of alkali (0.1 per cent.) or by boiling the solution. Care must be taken not to overlook this curious phenomenon when analysing worts and sugars.

In some breweries it is the custom to make daily records of the optical activity of the worts, and the practice is an excellent one. If, however, the indications which such a system is capable of affording are to be fully utilised, it is essential that the observations be made with regularity, in which case any change in character of material used, variation in mashing or sparging heats,

may at once be detected. Some brewers have adopted the practice, and have learnt its value, but in many establishments the records are not sufficiently systematic to be of much service.

The wort to be examined may be taken either from the mash-tun taps (in which case two samples should be taken, one 10 minutes after starting taps, the other after the sparge liquor has got well through the goods), or the wort as collected in the copper may be examined. When, however, brewers use sugar they generally dissolve some or the whole of it during the collection of the wort, so that if a copper sample is polarised, the calculation is complicated by the necessity for a correction for the sugar which is present. For that reason worts as running from taps are usually examined.

It is desirable to bring the worts to one definite gravity before polarising, for as results are always expressed in dry solids, and as the common solution factor 3.86 is used for that purpose, the amount of apparent dry solids and the corresponding angle will be altered by the differing gravities of the worts used. A good practice is to dilute the worts to about 1050 sp. gr. before ascertaining their rotatory power.

Whilst the polarimeter is of value where simple brewing methods are adopted, it is manifestly of much greater service where raw-grain conversion or boiled or decoction mashing systems are adopted, as by its means some knowledge of the composition of worts may be rapidly obtained.

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## CHAPTER XV.

STANDARD SOLUTIONS AND  
REAGENTS.

It is hardly necessary to say that the preparation of standard solutions requires much care, since it is sufficiently apparent that if they be not correct, analyses conducted by their means cannot be accurate, however much attention be paid to detail in the after operations.

Such solutions are best preserved in well-stoppered bottles, and, for most purposes, those of a capacity of 40 oz. will be found most useful, as they hold 1 litre—a quantity which may be conveniently made up of each standard.

Some solutions—silver nitrate, for example—are best kept in bottles made of dark blue glass, but others may be stored in bottles of either white or but slightly coloured material.

All such bottles should have clearly marked upon them the name and strength of the particular standard they contain, together with its reactionary power, thus : “Decinormal Iodine Solution (1 c.c. = 0.0032  $\text{SO}_2$ ).”

There is at times some difficulty in laboratories where there is much steam, from the tendency which the labels on bottles have to become detached. This may be obviated by first gumming on the labels in the ordinary manner, then painting them over, by means of a small brush,

with a mixture of two parts of ordinary size to one part of boiling water. This is allowed to dry (if necessary the sizing process may be repeated), and afterwards varnished over. Labels so affixed will last for a very long time without falling off or becoming erased.

When standard solutions which have not been very recently used are taken down from the laboratory shelf, a small quantity of condensed aqueous vapour will usually have collected on the inner surface of the bottle above the level of the liquid. Before using such solution it should be shaken, because the loss of water from the bulk of solution thus produced must render it slightly too strong—indeed, it is always well to shake the bottle before taking out any portion of the standard.

### Normal Sulphuric Acid. (N)

40 grammes per litre.

1 c.c. = 0.053  $\text{Na}_2\text{CO}_3$ .

1 c.c. = 0.090 Protein.

This is prepared by diluting about 30 c.c. of pure concentrated sulphuric acid to 1 litre, pouring the acid in a thin stream into distilled water, cooling, and making up to bulk. This solution will be a little too strong, and its exact value must therefore be ascertained and a calculation made of the dilution necessary in order to bring it to normal strength.

This may be accomplished by several methods. The easiest is that of weighing out an exact quantity of pure anhydrous sodic carbonate.\* For this purpose take, say,

\* T. E. Thorpe.

2 grammes of the pure salt named—which should have been recently heated—and place it in a tared platinum dish, dissolve in a small quantity of water, covering with a clean watch-glass. Temporarily remove the watch-glass and run in exactly 25 c.c. of the sulphuric acid to be standardised, and immediately replace the cover. When the effervescence at first occurring has ceased, place the dish on the water bath and evaporate the contents to complete dryness, previously rinsing in any drops of liquid which may have collected on the inner surface of the watch-glass.

Now heat to  $350^{\circ}$  F. in an air bath, cool under desiccator, and weigh, repeating this until constant.

From this we may easily calculate the exact strength of our solution. The  $\text{CO}_2$  in the sodic carbonate has been displaced by  $\text{SO}_3$ , and, as the atomic weight of the former is 44 as against 80 for the latter, it is evident that the increased weight of the residue over that of the sodic carbonate originally taken is proportional to the amount of sulphuric acid employed, provided there be present an excess of the salt named, and the quantity recommended ensures this being so.

An actual example will make the calculation clear:—  
A platinum dish was carefully tared, and about 2 grammes of pure anhydrous sodic carbonate was added, the weights being as follows:—

|                                 |     |     |     | Grammes. |
|---------------------------------|-----|-----|-----|----------|
| Dish + $\text{Na}_2\text{CO}_3$ | ... | ... | ... | 65.306   |
| Dish ...                        | ... | ... | ... | 63.214   |
| $\text{Na}_2\text{CO}_3$ taken  | ... | ... | ... | 2.092    |

25 c.c. of the sulphuric acid of approximately normal strength was then run in, and the whole evaporated to dryness, heated to  $350^{\circ}$  F. for some time, cooled, and weighed. The dish and contents now weighed 65.779 grammes, an increase of 0.473 gramme.

Now this increase is evidently in proportion to the difference between the molecular weights of  $\text{CO}_2$  and  $\text{SO}_3$ , that is, 44 of  $\text{CO}_2$  will be replaced by 80 of  $\text{SO}_3$ ; or the presence of 80 parts of  $\text{SO}_3$  will give us an increased weight of 36. Therefore,  $36 : 80 :: 0.473 = 1.051$  grammes of  $\text{SO}_3$  in 25 c.c. of dilute acid; or 4.204 per 100 c.c., in place of 4.00, the correct quantity. We may now dilute this as follows:— $4.00 : 100 :: 4.204 : 105.1$ . That is, each 100 c.c. must be diluted to 105.1 c.c. to make it of correct strength, or to each litre we may add 51 c.c. The accuracy of this may be verified by making another experiment with the diluted liquid, either by evaporation with sodic carbonate as before, or by measuring out 2 c.c., diluting, precipitating with baric chloride, filtering, and calculating from the weight of precipitate the  $\text{SO}_3$  in the solution.

### Normal Sodium Hydrate. (N)

40 grammes per litre.

1 c.c. = 0.06 acetic acid.

1 c.c. = 0.09 lactic acid.

Normal alkali may be of either caustic soda or ammonia. The former, however, is most generally useful, as the latter, from its volatile nature, constantly becomes weaker, and therefore requires frequent re-standardising.

Normal soda is prepared by dissolving 45 to 50 grammes



of sodium hydrate in one litre of water, titrating the solution against normal acid, calculating therefrom its exact strength and the dilution necessary to reduce it to 40 grammes of actual NaHO per litre.

### **Decinormal Acid and Alkali. (N/10)**

These solutions are prepared by measuring 100 c.c. of the normal solution into a litre flask and diluting to mark with distilled water.

It need hardly be remarked that all such measurements must be conducted with the greatest care, and that the water used for effecting dilution should be perfectly pure, recently distilled, and at a temperature of 60° F.

### **Seminormal Ammonia. (N/2)**

8.5 grammes  $\text{NH}_3$  per litre.

1 c.c. = 0.030 acetic acid.

1 c.c. = 0.045 lactic acid.

Standard ammonia is sometimes used in preference to standard soda. If made up to normal strength, the standard is very liable to become weak, owing to evaporation of the gas at ordinary temperatures. A seminormal (half normal) solution, however, keeps well if placed in a cool place and in a well-stoppered bottle.

To prepare this standard, take about 32 c.c. of strong ammonium hydrate (sp. gr. 0.880), dilute to 1 litre, and standardise against the normal acid, 2 c.c. of seminormal ammonia being equal to 1 c.c. of normal acid.

### **Decinormal Ammonia. (N/10)**

This standard is made by taking 200 c.c. of seminormal

ammonia, and diluting to 1 litre with distilled water. The solution keeps its strength well if stored in well-stoppered bottles, but all ammonia standards should be titrated from time to time against the corresponding acid standard in order to ascertain their correct value.

### **Four-times Normal Acid and Alkali. (4N)**

These solutions are employed chiefly for the purposes of inducing the inversion of the several carbohydrates, and it is not necessary that they should be absolutely correct. The acid employed in this case is hydrochloric. 400 c.c. of strong acid is diluted to 1 litre, titrated against normal alkali, and appropriately diluted; it being remembered that each cubic centimetre of the corrected standard should require 4 c.c. of normal alkali. The corresponding four-times normal alkali is made by dissolving about 160 grammes of sodium hydrate, diluting to 1 litre, and standardising against the acid previously prepared.

### **Decinormal Potassium Permanganate. (N/10)**

3·156 grammes per litre.

1 c.c. = 0·0056 Fe.

1 c.c. = 0·0080  $\text{Fe}_2\text{O}_3$ .

1 c.c. = 0·0017  $\text{H}_2\text{S}$ .

1 c.c. = 0·0056 CaO.

Pure crystals of the salt are taken. Exactly 3·156 grammes is weighed out, dissolved in freshly-prepared distilled water, and diluted to 1 litre. If kept in a closely-stoppered bottle—preferably of blue glass—this solution will retain its strength for many months. If the exact quantity of

the salt be weighed out, it is essential that it be perfectly pure and dry, and such a preparation can be obtained only of the best manufacturers.

If the salt employed be not absolutely dry—*pure* it must be, or the standard will soon decompose—the solution is titrated against some suitable salt to verify its exact strength. For this purpose ferrous ammonium sulphate ( $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ ) is well suited, being a perfectly stable salt when pure, and containing exactly one-seventh of its weight of iron. To titrate the permanganate, weigh out exactly 0.7 gramme of the salt named, dissolve in distilled water in a flask, add 5 c.c. of dilute sulphuric acid, and run in the permanganate from a burette until the pink colour becomes permanent. If accurate, it should have required exactly 17.8 c.c. of permanganate.

### **Permanganate of Potash for Forschammer Oxygen Process.**

1 c.c. contains 0.0001 gramme of available oxygen.

Dissolve 0.395 gramme of the pure, dry salt in 1 litre of recently-prepared distilled water.

### **Sodium Hyposulphite for Forschammer Process.**

Two grammes of the pure crystallised salt per litre of distilled water.

### **Potassium Iodide Solution for Forschammer Process.**

Ten grammes of the salt is dissolved, and made up to 100 c.c. with distilled water.

**Decinormal Iodine. (N/10)**

12·7 grammes per litre.

1 c.c. = 0·0032 gramme  $\text{SO}_2$ .

Re-sublimed iodine must be employed for this purpose. This may generally be procured of sufficient purity ; but if not, the fairly pure iodine is intimately mixed with one-fourth its weight of pure potassium iodide, placed in a large watch-glass, and gently heated. Over it is placed an inverted funnel, the tube of which is closed with a piece of glass rod and small india-rubber tubing. The iodine sublimes in beautiful crystals, and is perfectly pure.

12·7 grammes of this is now carefully weighed out and, together with 20 grammes of pure potassium iodide, is dissolved in a little water, then diluted to 1 litre, when the solution will be strictly decinormal. The liquid must not be heated so as to promote solution, or the vapours of iodine may be lost.

**Decinormal Sodium Hyposulphite. (N/10)**

24·8 grammes of crystallised salt per litre.

Sodium hyposulphite may now be obtained of almost perfect purity, so that the quantity specified may be directly weighed out, dissolved, and diluted to 1 litre. It should correspond, volume for volume, with the iodine solution already described. The solution is, unfortunately, somewhat prone to decomposition, depositing sulphur, especially if exposed to the light. This tendency may, according to Mohr, be prevented by the addition of a little sesqui-

carbonate of ammonia to the solution—2 grammes per litre is sufficient.\*

### Soluble Starch.

Take pure potato starch and cover with dilute hydrochloric acid of specific gravity 1037°. Allow to stand for a week at 60° F. The operation is best carried out in a Winchester quart bottle containing 1 lb. of starch and 1000 c.c. of diluted acid.

The starch is washed with tap water by repeated decantation, using distilled water for the final washings until these are perfectly free from acid. If the slightest trace of acid is left, the starch will be useless.

When the washing is completed, drain off the water, best done by collecting the starch on a filter paper fitted into a Buchner's funnel and pumping as dry as possible.

The starch is finally dried at a gentle heat not exceeding 110° F. as quickly as possible, powdered in a mortar and placed in a bottle or tin.

### Silver Nitrate.

1 c.c. = 0.001 gramme Cl, or 0.00165 NaCl.

Dissolve 4.79 grammes of pure recrystallised silver nitrate in distilled water and dilute to 1 litre.

### Indigo Solution.

1 c.c. = 0.0001 gramme N.

1 „ = 0.000386 „  $N_2O_5$ .

\* O'Shaughnessy has stated (*Analyst*, 1898, p. 118) that the addition of salicylic acid entirely prevents the decomposition of the solution.

Take 4 grammes of indigo carmine, place in a small beaker, and mix with a few drops of cold water to form a paste; then add 4 c.c. of strong pure sulphuric acid, cover beaker with a watch-glass, and allow to stand over-night. Now dilute with water, thoroughly mix, and make up to a litre, filtering if necessary.

This solution keeps for a long time.

Standardise solution by titration against a standard solution of potassium nitrate, prepared as below, and diluted until 5 c.c. of the potassium nitrate solution requires exactly 7 c.c. of the indigo. The titration is performed in a flask or porcelain dish with 5 c.c. of the nitrate solution, previously diluted to 50 c.c. with distilled water. Strong sulphuric acid is run in, and the operation conducted precisely as described under "Water Analysis."

### Potassium Nitrate.

1 c.c. = 0.14 milligramme N.

1.011 gramme of the crystallised salt is dissolved in distilled water and made up to 1 litre.

### Iron Solution.

1 c.c. = 0.1 milligramme Fe.

0.7 gramme ferrous ammonium sulphate is weighed out, dissolved in about 250 c.c. of distilled water, a little bromine water added, and the solution boiled in a flask for 5 or 10 minutes to oxidise the iron into the ferric state. The solution is then cooled, transferred to a litre flask, and diluted to mark.

### Nessler's Test.

This consists of a solution of iodide of potassium saturated with periodide of mercury, and rendered powerfully alkaline with potash or soda. Weigh out 35 grammes of iodide of potassium and 13 grammes of mercuric chloride (corrosive sublimate), and dissolve it in about 800 c.c. of water. Now add a cold saturated solution of mercuric chloride until a permanent precipitate is produced; render this liquid alkaline by adding 120 grammes of caustic soda, and dilute to 1 litre. To sensitise the solution, it is finally mixed with a little more of the cold mercuric chloride solution and allowed to settle. The solution is kept in a bottle with some of the precipitate at the bottom. Nessler's test should have a slightly yellow tint. If colourless, it is sure not to be sensitive.

### Dilute Standard Solution of Ammonia.

Weigh up very carefully 0.0315 gramme of chloride of ammonium—the sal-ammoniac of commerce in dry fibrous crystals answers well—place in a litre flask, dissolve in water free from ammonia, and dilute to mark with water free from ammonia. Each c.c. = 0.01 milligramme of  $\text{NH}_3$ . Wanklyn recommends the use of a stronger solution than this (0.1 milligramme  $\text{NH}_3$ ), but the strength named is of more general service.

### Alkaline Permanganate of Potash.

This is made by dissolving 200 grammes of stick caustic potash\* with 8 grammes of crystallised potassic permanganate

\* Do not use caustic soda, for it generally contains traces of ammonia, which are most difficult to remove.



in about 500 c.c. of water and evaporating in a large porcelain dish almost to dryness. This effectually expels all traces of ammonia which may have been present, and the liquid has now merely to be cooled to 60° F. and diluted to 1 litre with water perfectly free from ammonia, prepared as described below.

It is necessary to make a blank distillation experiment with the alkaline permanganate in order to ascertain correction, if any, to be made. In practice, it is not difficult to prepare the solution so that the ammonia derived from it would not amount when expressed in a water analysis to more than 0.005 parts per million, and such a correction may be neglected.

### **Water Free from Ammonia.**

Distilled water as usually obtained contains considerable quantities of ammonia, and therefore must not be employed for Nesslerising. It is easy to expel ammonia from distilled water by vigorous boiling in a capacious flask for about half an hour, afterwards cooling and transferring to bottle. Unfortunately, however, boiling water attacks glass,\* and for this reason when prepared as above is slightly cloudy. Satisfactory distilled water may be prepared by boiling ordinary tap water in the apparatus used for the distillation of water in the ammonia process, rejecting such distillate until a portion of it gives no colour with Nessler's reagent, or the water in retort may be acidulated with pure sulphuric acid, when the whole distillate will be free from ammonia.

\* Fresenius found that 1 litre of distilled water dissolves, by protracted boiling in a glass flask, about 14 milligrammes of the constituents of the glass.

## Preparation of Litmus Solution and Paper.

For certain titration purposes, a pure solution of litmus is required. That ordinarily obtained by a simple infusion of litmus is not sufficiently delicate. A solution of great sensitiveness may be made as follows:—Reduce the litmus itself to a powder, place it in a flask, pour on a little strong alcohol of about 80 per cent., and boil. Throw away the liquid, and repeat this operation twice. Then digest the litmus thus purified with cold distilled water for some time, decant off, wash with more water until but little further colour is extracted; let the liquid settle bright—it is difficult to filter—decant, and add a little sulphuric acid until the solution is of a brilliant red. Heat to boiling, and afterwards add baryta water until the red colour changes to violet, the sulphuric acid being removed and precipitated as baric sulphate. Now set aside, and when the supernatant liquid is perfectly clear, decant it off. Litmus paper may be made from this by painting sheets of unglazed paper with the solution, allowing to dry in a room perfectly free from acid or other fumes, afterwards cutting into strips of suitable sizes. The paper best suited for this purpose is the unglazed paper used by printers. If a glazed paper be used, it must first be washed with a very dilute solution of sodic hydrate, to neutralise the acid reaction normal to all such paper.

It is a very common practice to employ filtering-paper; but papers so prepared are scarcely so delicate or sharp in reaction as those prepared in the manner described.

### **Methyl Orange.**

Dissolve 0.2 gramme of the substance in 100 c.c. of distilled water. This indicator is unaffected by carbonic acid or sulphuretted hydrogen, but it must not be used for organic acids. The titration should be conducted in cold, not in warm, solutions. It gives a yellow colour with alkalies, pink with acids.

### **Phenolphthalein.**

Dissolve 0.25 gramme in 100 c.c. of strong alcohol, then gradually mix with an equal bulk of distilled water. This indicator is extremely delicate, and is most useful in titrating organic acids, but useless for the titration of ammonia or for alkalies in the presence of ammonia. It is colourless in a neutral or acid liquid, but red in the presence of an alkali.

### **Aluminium Hydrate.**

Take 50 grammes of common potash alum, dissolve it in about a litre of water, and add cautiously a solution of sodium hydrate until the liquid is faintly alkaline, allow to subside, decant off, and throw away the supernatant liquid; add more water, stir up well, allow to subside, and again decant, repeating this until the liquid is no longer alkaline. Now add sufficient water to the precipitated aluminium hydrate to form, when the whole is stirred up, a mixture of such a fluidity that it will readily pour out of any vessel; transfer to storage bottle, shaking up each time before using, or, if preferred, moist aluminic hydrate may be purchased and worked up with water to the consistency of a thin cream.

### Indicator for Diastatic Power.\*

One gramme of ferrous ammonium sulphate and the same quantity of ammonium thiocyanate are dissolved in 10 c.c. of water at a moderate temperature, say at 120° F., and immediately cooled; 5 c.c. concentrated hydrochloric acid are then added. The solution so obtained has invariably a brownish-red colour, due to the presence of ferric salt, which latter must be reduced. For this purpose zinc dust is the most satisfactory reagent to employ, and a mere trace is sufficient to decolorise the solution if pure reagents have been employed.

When kept for some hours the indicator develops the red coloration by atmospheric oxidation. It may, however, be decolorised by the addition of a further quantity of zinc dust, but its delicacy is decreased after it has been decolorised several times. For practical purposes the indicator may be too delicate, and it is recommended to prepare it the day before it is required for use, as it gives the best results after the second decolorisation.

### Reagents for Nitrous Acid Test.

*Sulphanilic Acid*.—Dissolve 1 gramme of the salt in 100 c.c. of hot water. The solution will be found to keep well.

*Naphthylamine Hydrochloride*.—Boil 100 c.c. of water with  $\frac{1}{2}$  gramme of the salt for 10 minutes, keeping the volume fairly constant. The solution tends to become

\* Taken from Report of Malt Analysis Committee, *Journ. Inst. Brew.*, 1906, p. 9.

slightly pink on standing, but not sufficiently so as to interfere with its use.

*Standard Solution of Sodium Nitrite.*—This salt is seldom pure and is very deliquescent ; it is therefore desirable to prepare the silver salt.

To a cold solution of commercial sodium or potassium nitrite add a solution of silver nitrate as long as a precipitate appears. Decant the clear liquid and thoroughly wash the precipitate with cold water. Finally dissolve this in boiling water, concentrate and crystallise the silver nitrite from the salt solution. Dry in the dark at the ordinary temperature, using a vacuum if possible, and store in a dark bottle.

Weigh out 0.22 gramme of the dry silver nitrite and dissolve in a little hot water. Decompose this with a slight excess of sodium chloride solution, cool, and dilute to 1 litre. Allow the precipitated silver chloride to settle. Remove 5 c.c. of the clear solution with a pipette and dilute the same to 1 litre.

This second solution, which is the standard solution to be used, will now contain an amount of nitrite equal to 0.0001 milligramme of nitrogen in each cubic centimetre.

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## APPENDIX.

Names of the more Common Elementary Substances, with their  
Symbols and Atomic Weights.

| Name.             | Symbol. | Atomic weight. |
|-------------------|---------|----------------|
| Aluminium ... ..  | Al      | 27·1           |
| Arsenic ... ..    | As      | 74·96          |
| Barium ... ..     | Ba      | 137·37         |
| Bromine ... ..    | Br      | 79·92          |
| Calcium ... ..    | Ca      | 40·09          |
| Carbon ... ..     | C       | 12·00          |
| Chlorine ... ..   | Cl      | 35·46          |
| Chromium ... ..   | Cr      | 52·00          |
| Copper ... ..     | Cu      | 63·57          |
| Hydrogen ... ..   | H       | 1·008          |
| Iodine ... ..     | I       | 126·92         |
| Iron ... ..       | Fe      | 55·85          |
| Lead ... ..       | Pb      | 207·10         |
| Magnesium ... ..  | Mg      | 24·32          |
| Manganese ... ..  | Mn      | 54·93          |
| Mercury ... ..    | Hg      | 200·00         |
| Nitrogen ... ..   | N       | 14·01          |
| Oxygen ... ..     | O       | 16·00          |
| Phosphorus ... .. | P       | 31·04          |
| Platinum ... ..   | Pt      | 195·20         |
| Potassium ... ..  | K       | 39·10          |
| Silver ... ..     | Ag      | 107·88         |
| Sodium ... ..     | Na      | 23·00          |
| Sulphur ... ..    | S       | 32·07          |
| Tin ... ..        | Sn      | 119·00         |
| Zinc ... ..       | Zn      | 65·37          |

The above figures are taken from the table of International Atomic Weights, 1911.

### Conversion of Thermometer Degrees.

° C. to ° F., multiply by 9, divide by 5, then add 32.

° F. to ° C., first subtract 32, then multiply by 5, and divide by 9.

## Relations of Measures to Weights.

|                       |                   |                                   |
|-----------------------|-------------------|-----------------------------------|
| 1 minim               | is the measure of | 0.91 grain of water.              |
| 1 fluid drachm        | „                 | 54.68 grains „                    |
| 1 fluid ounce         | „                 | 1 ounce or 437.5 grains of water. |
| 1 pint                | „                 | 1.25 pounds or 8,750 „            |
| 1 gallon              | „                 | 10 pounds or 70,000 „             |
| 1 cubic inch of water | at 60° F. weighs  | 252.458 grains.                   |
| 1 „ „                 | „ „               | 0.577047 ounce.                   |
| 1 „ „                 | „ „               | 16.359 grammes.                   |
| 1 cubic foot          | „ „               | 997.137 ounces.                   |
| 1 „ „                 | „ „               | 62.321 pounds.                    |
| 1 „ „                 | „ „               | 28.2684 kilogrammes.              |

## Table for Conversion of Various Measures.

| To convert               | Into                     | Multiply by |
|--------------------------|--------------------------|-------------|
| Grammes ... ..           | Grains ... ..            | 15.432      |
| „ ... ..                 | Ounces (Avoirdupois) ... | 0.035273    |
| Kilogrammes ... ..       | Pounds ... ..            | 2.2046      |
| Grains ... ..            | Grammes ... ..           | 0.0648      |
| Ounces (Avoirdupois) ... | „ ... ..                 | 28.3495     |
| Pounds ... ..            | Kilogrammes ... ..       | 0.45359     |
| Litres ... ..            | Fluid ounces ... ..      | 35.2154     |
| „ ... ..                 | Pints ... ..             | 1.76077     |
| „ ... ..                 | Gallons ... ..           | 0.22        |
| Fluid ounces ... ..      | Cubic centimetres ...    | 28.396      |
| Pints ... ..             | „ „ ... ..               | 567.92      |
| „ ... ..                 | Litres ... ..            | 0.56792     |
| „ ... ..                 | Cubic inches ... ..      | 34.659      |
| Gallons ... ..           | Litres ... ..            | 4.5434      |
| „ ... ..                 | Cubic inches ... ..      | 277.273     |
| „ ... ..                 | „ fect ... ..            | 0.16        |
| Bushels ... ..           | „ „ ... ..               | 1.28        |
| Cubic inches ... ..      | „ centimetres ...        | 16.386      |
| „ ... ..                 | Fluid ounces ... ..      | 0.57704     |
| „ ... ..                 | Pints ... ..             | 0.028852    |
| Cubic feet ... ..        | Litres ... ..            | 28.3153     |
| „ ... ..                 | Gallons ... ..           | 6.2321      |
| „ ... ..                 | Bushels ... ..           | 0.78        |
| Parts per 100,000 ... .. | Grains per gallon ...    | 0.7         |
| Grains per gallon ... .. | Parts per 100,000 ...    | 1.4286      |
| Grammes per litre ... .. | Grains per gallon ...    | 70.0        |
| Grains per gallon .. ..  | Grammes per litre ...    | 0.014286    |
| Grammes per fluid drachm | Grains per fluid ounce   | 123.46      |



**Metric System.***Weights.*

|               |   |                             |
|---------------|---|-----------------------------|
| 1 milligramme | = the thousandth part of a gramme, or   | 0·001 gramme.               |
| 1 centigramme | = the hundredth                   ,,                   ,,                                 | 0·01                   ,,   |
| 1 decigramme  | = the tenth                           ,,                   ,,                             | 0·1                   ,,    |
| 1 gramme      | = the weight of 1 cubic centimetre<br>of water at 4° C.                   ...             | 1·0                   ,,    |
| 1 decagramme  | =                   ...                   ...                   ...                   ... | 10·0 grammes.               |
| 1 hectogramme | =                   ...                   ...                   ...                   ... | 100·0                   ,,  |
| 1 kilogramme  | =                   ...                   ...                   ...                   ... | 1000·0                   ,, |

*Measures of Capacity.*

|              |   |   |
|--------------|---|---|
| 1 millilitre | = | 1 cubic centimetre, or the measure of 1 gramme<br>of water at 4° C. |
| 1 centilitre | = | 10 cubic centimetres.   |
| 1 decilitre  | = | 100                   ,,                   ,,                       |
| 1 litre      | = | 1000                   ,,                   ,,                      |
| 1 decalitre  | = | 10 litres.  |
| 1 hectolitre | = | 100                   ,,  |
| 1 kilolitre  | = | 1000                   ,,   |

*Measures of Length.*

|              |   |   |
|--------------|---|---|
| 1 millimetre | = | 0·001 metre.  |
| 1 centimetre | = | 0·01                   ,,                                       |
| 1 decimetre  | = | 0·1                   ,,  |
| 1 metre      | = | the ten-millionth part of a quarter of the earth's<br>meridian. |
| 1 decametre  | = | 10 metres.  |
| 1 hectometre | = | 100                   ,,  |
| 1 kilometre  | = | 1000                   ,,                                       |

*Weights.*

|             | In English<br>Grains. | In Troy<br>Ounces. | In<br>Avoirdupois<br>Drachins. | In<br>Avoirdupois<br>Pounds. |
|-------------|-----------------------|--------------------|--------------------------------|------------------------------|
| Milligramme | 0·01543               | 0·000032           | 0·0005644                      | 0·0000022                    |
| Centigramme | 0·15432               | 0·000322           | 0·0056438                      | 0·0000220                    |
| Decigramme  | 1·54323               | 0·003215           | 0·0564378                      | 0·0002205                    |
| Gramme      | 15·43235              | 0·032151           | 0·5643776                      | 0·0022046                    |

1 grain = 0·064799 gramme.   1 troy ounce = 31·103496 grammes.  
 1 lb. avoird. = 0·453593 kilogramme.   1 cwt. = 50·802377 kilo-  
 grammes.

*Measures of Capacity.*

|                             | In Cubic<br>Inches. | In Cubic<br>Feet. | In<br>Pints. | In<br>Gallons. | In<br>Bushels. |
|-----------------------------|---------------------|-------------------|--------------|----------------|----------------|
| Millilitre or<br>cub. cent. | 0·06103             | 0·000035          | 0·00176      | 0·0002201      | 0·0000275      |
| Litre or cub.<br>decim.     | 61·02705            | 0·035317          | 1·76077      | 0·2200967      | 0·0275121      |

**Gauging Calculations.***Round Vessels.*

To find contents :—Square diameter in inches, and multiply by 0·7854 = area.

Area  $\times$  0·00283257 = gallons per inch.

Multiply this result by depth (in inches), and result equals content.

Area  $\times$  0·00035407 = bushels per inch.

Multiply by depth as before, and result equals content in bushels.

To find circumference, multiply diameter by 3·1416.

To find diameter from circumference, multiply it by 0·31831.

Radius equals one-half of diameter.

*Square or Right-angled Vessels.*

Multiply width by length and by depth.

Multiply result in inches by 0·0036065 = gallons.

Multiply result in inches by 0·004508 = bushels.

**Influence of Pressure on Boiling-point of Water.**

Water boils at a pressure of—

|                  |     |     |     |     | F.  |
|------------------|-----|-----|-----|-----|-----|
| 2 atmospheres at | ... | ... | ... | ... | 248 |
| 3        ,,      | ... | ... | ... | ... | 275 |
| 4        ,,      | ... | ... | ... | ... | 293 |
| 8        ,,      | ... | ... | ... | ... | 338 |
| 16       ,,      | ... | ... | ... | ... | 392 |

## Influence of Vacuum on Boiling-point of Water.

|   |   |   |                 |   | ° F. |
|---|---|---|-----------------|---|------|
| Temperature of boiling-point of water in air is ... |   |   |                 |   | 212  |
| "   | " | " | 5 in. vacuum is |   | 195  |
| "   | " | " | 10              | " | 185  |
| "   | " | " | 15              | " | 160  |
| "   | " | " | 20              | " | 150  |
| "   | " | " | 25              | " | 130  |
| "   | " | " | 26              | " | 120  |
| "   | " | " | 27              | " | 112  |
| "   | " | " | 28              | " | 100  |
| "   | " | " | 29              | " | 72   |
| "   | " | " | 29½             | " | 52   |

## Temperature of Steam under Pressure.

| Pressure per<br>Square Inch. | Temperature. | Pressure per<br>Square Inch. | Temperature. |
|------------------------------|--------------|------------------------------|--------------|
| Lbs.                         | ° F.         | Lbs.                         | ° F.         |
| 0·0                          | 212·0        | 55·3                         | 302·9        |
| 0·3                          | 213·1        | 60·3                         | 307·5        |
| 2·3                          | 219·6        | 65·3                         | 312·0        |
| 4·3                          | 225·3        | 70·3                         | 316·1        |
| 6·3                          | 230·6        | 75·3                         | 320·2        |
| 8·3                          | 235·5        | 80·3                         | 324·1        |
| 10·3                         | 240·1        | 85·3                         | 327·9        |
| 15·3                         | 250·4        | 95·3                         | 334·6        |
| 20·3                         | 259·3        | 105·3                        | 341·1        |
| 25·3                         | 267·3        | 115·3                        | 347·2        |
| 30·3                         | 274·4        | 125·3                        | 352·9        |
| 35·3                         | 281·0        | 145·3                        | 363·4        |
| 40·3                         | 287·1        | 165·3                        | 372·9        |
| 45·3                         | 292·7        | 185·3                        | 381·7        |
| 50·3                         | 298·0        | 235·3                        | 401·1        |

Kopp's Table, showing the Expansion of Water from  
 $0^{\circ}\text{C.}$  to  $100^{\circ}\text{C.}$  ( $32^{\circ}\text{F.}$  to  $212^{\circ}\text{F.}$ ).

| Temperature.        |                     | Volume.  | Temperature.        |                     | Volume.  |
|---------------------|---------------------|----------|---------------------|---------------------|----------|
| $^{\circ}\text{C.}$ | $^{\circ}\text{F.}$ |          | $^{\circ}\text{C.}$ | $^{\circ}\text{F.}$ |          |
| 0                   | 32.0                | 1.000000 | 21                  | 69.8                | 1.001776 |
| 1                   | 33.8                | 0.999947 | 22                  | 71.6                | 1.001995 |
| 2                   | 35.6                | 0.999908 | 23                  | 73.4                | 1.002225 |
| 3                   | 37.4                | 0.999885 | 24                  | 75.2                | 1.002465 |
| 4                   | 39.2                | 0.999877 | 25                  | 77.0                | 1.002715 |
| 5                   | 41.0                | 0.999883 | 30                  | 86.0                | 1.004064 |
| 6                   | 42.8                | 0.999903 | 35                  | 95.0                | 1.005697 |
| 7                   | 44.6                | 0.999938 | 40                  | 104.0               | 1.007531 |
| 8                   | 46.4                | 0.999986 | 45                  | 113.0               | 1.009541 |
| 9                   | 48.2                | 1.000048 | 50                  | 122.0               | 1.011766 |
| 10                  | 50.0                | 1.000124 | 55                  | 131.0               | 1.014100 |
| 11                  | 51.8                | 1.000213 | 60                  | 140.0               | 1.016590 |
| 12                  | 53.6                | 1.000314 | 65                  | 149.0               | 1.019302 |
| 13                  | 55.4                | 1.000429 | 70                  | 158.0               | 1.022246 |
| 14                  | 57.2                | 1.000556 | 75                  | 167.0               | 1.025440 |
| 15                  | 59.0                | 1.000695 | 80                  | 176.0               | 1.028581 |
| 16                  | 60.8                | 1.000846 | 85                  | 185.0               | 1.031894 |
| 17                  | 62.6                | 1.001010 | 90                  | 194.0               | 1.035397 |
| 18                  | 64.4                | 1.001184 | 95                  | 203.0               | 1.039094 |
| 19                  | 66.2                | 1.001370 | 100                 | 212.0               | 1.042986 |
| 20                  | 68.0                | 1.001567 |                     |                     |          |

Table showing the Quantity of Sugar in Pounds Avoirdupois contained at various Degrees of Specific Gravity, at 60° Fahr.

| Specific Gravity. | Lbs. per Gallon. | Specific Gravity. | Lbs. per Gallon. | Specific Gravity. | Lbs. per Gallon. |
|-------------------|------------------|-------------------|------------------|-------------------|------------------|
| 1.000             | 0.0000           | 1.099             | 2.6130           | 1.200             | 5.2901           |
| 1.005             | 0.1275           | 1.104             | 2.7446           | 1.205             | 5.4203           |
| 1.010             | 0.2550           | 1.109             | 2.8740           | 1.210             | 5.5506           |
| 1.015             | 0.3825           | 1.111             | 2.9263           | 1.215             | 5.6942           |
| 1.020             | 0.5100           | 1.116             | 3.0563           | 1.221             | 5.8680           |
| 1.025             | 0.6355           | 1.121             | 3.1871           | 1.228             | 6.0642           |
| 1.030             | 0.7610           | 1.126             | 3.3174           | 1.233             | 6.2012           |
| 1.035             | 0.8866           | 1.131             | 3.4490           | 1.238             | 6.3362           |
| 1.037             | 0.9449           | 1.136             | 3.5882           | 1.243             | 6.4650           |
| 1.042             | 1.0606           | 1.141             | 3.7281           | 1.248             | 6.5903           |
| 1.047             | 1.2171           | 1.146             | 3.8677           | 1.253             | 6.7240           |
| 1.052             | 1.3472           | 1.151             | 4.0070           | 1.258             | 6.8643           |
| 1.057             | 1.4802           | 1.156             | 4.1319           | 1.266             | 7.1060           |
| 1.062             | 1.6142           | 1.161             | 4.2771           | 1.271             | 7.2601           |
| 1.067             | 1.7496           | 1.166             | 4.4115           | 1.276             | 7.4109           |
| 1.072             | 1.8843           | 1.171             | 4.5460           | 1.281             | 7.5600           |
| 1.074             | 1.9385           | 1.176             | 4.6764           | 1.286             | 7.7048           |
| 1.079             | 2.0734           | 1.181             | 4.8051           | 1.291             | 7.8482           |
| 1.084             | 2.2080           | 1.186             | 4.9300           | 1.296             | 7.9879           |
| 1.089             | 2.3438           | 1.190             | 5.0304           | 1.300             | 8.1001           |
| 1.094             | 2.4792           | 1.195             | 5.1602           |                   |                  |

Table for Correction of Specific Gravity for Variation of Temperature.

| Specific Gravity. | Temperature. |     |     |     |     |     |     |     |      |      |      |      |      |      |      |
|-------------------|--------------|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|------|------|------|
|                   | 60°          | 65° | 70° | 75° | 80° | 85° | 90° | 95° | 100° | 105° | 110° | 115° | 120° | 125° | 130° |
| 1000              | —            | 0·4 | 1·0 | 1·6 | 2·3 | 2·8 | 3·6 | 4·3 | 5·2  | 6·1  | 7·1  | 8·0  | 9·1  | 10·2 | 11·4 |
| 10                | —            | 0·4 | 1·1 | 1·6 | 2·3 | 2·9 | 3·7 | 4·4 | 5·4  | 6·3  | 7·3  | 8·3  | 9·4  | 10·5 | 11·7 |
| 20                | —            | 0·4 | 1·1 | 1·7 | 2·3 | 3·0 | 3·8 | 4·6 | 5·5  | 6·4  | 7·6  | 8·4  | 9·6  | 10·7 | 11·9 |
| 30                | —            | 0·4 | 1·1 | 1·7 | 2·4 | 3·1 | 3·9 | 4·7 | 5·6  | 6·6  | 7·6  | 8·4  | 9·8  | 10·9 | 12·2 |
| 40                | —            | 0·4 | 1·1 | 1·7 | 2·5 | 3·2 | 4·0 | 4·8 | 5·8  | 6·8  | 7·8  | 8·8  | 10·0 | 11·2 | 12·5 |
| 50                | —            | 0·6 | 1·2 | 1·8 | 2·5 | 3·3 | 4·1 | 5·0 | 5·9  | 6·9  | 8·0  | 9·0  | 10·3 | 11·4 | 12·8 |
| 60                | —            | 0·6 | 1·2 | 1·8 | 2·7 | 3·5 | 4·3 | 5·1 | 6·1  | 7·0  | 8·2  | 9·2  | 10·5 | 11·7 | 13·0 |
| 70                | —            | 0·7 | 1·3 | 1·9 | 2·7 | 3·5 | 4·4 | 5·3 | 6·3  | 7·3  | 8·4  | 9·5  | 10·8 | 12·0 | 13·3 |
| 80                | —            | 0·7 | 1·3 | 2·0 | 2·8 | 3·6 | 4·5 | 5·5 | 6·4  | 7·5  | 8·6  | 9·7  | 11·0 | 12·2 | 13·6 |
| 90                | —            | 0·7 | 1·3 | 2·0 | 2·9 | 3·7 | 4·6 | 5·6 | 6·6  | 7·7  | 8·8  | 10·0 | 11·3 | 12·5 | 13·9 |
| 1100              | —            | 0·7 | 1·4 | 2·1 | 2·9 | 3·8 | 4·7 | 5·7 | 6·8  | 7·9  | 9·0  | 10·2 | 11·5 | 12·8 | 14·2 |
| 10                | —            | 0·7 | 1·4 | 2·1 | 3·0 | 3·9 | 4·9 | 5·9 | 6·9  | 8·0  | 9·2  | 10·4 | 11·8 | 13·0 | 14·5 |
| 20                | —            | 0·7 | 1·4 | 2·3 | 3·1 | 4·0 | 5·0 | 6·0 | 7·1  | 8·2  | 9·4  | 10·6 | 12·0 | 13·3 | 14·8 |
| 30                | —            | 0·8 | 1·5 | 2·3 | 3·2 | 4·1 | 5·1 | 6·2 | 7·2  | 8·4  | 9·6  | 10·6 | 12·2 | 13·3 | 15·1 |

*In using this Table Add Correction for the Gravity.*

EXAMPLE.—A wort, weighed at 85°, showed a specific gravity of 1050. By reference to table, it will be seen that an addition of 3·3 must be made for that temperature, so that the wort at 60° would weigh 1053·3.

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